

7 October 1982

# The future of the test-tube baby

*Last week's row in Britain about in vitro fertilization is less demanding of attention than the need for all concerned to plan for what the future holds.*

Newspaper reports of a speech by Dr Robert Edwards to a bunch of deadline-conscious medical journalists seem once more to have stirred up the latent British willingness to believe the worst of those who work in some fields of research (see *Nature* 30 September, p.383). In the 1950s, there was a common view that a pair of horns growing out of the head was an essential qualification for a job in nuclear physics. More recently, the same sinister suppositions have, if briefly, attached to genetic manipulation by recombinant DNA. Dr Edwards, who, with the surgeon Mr Patrick Steptoe, has pioneered the techniques that now make it possible to fertilize human ova outside the body of the woman who is their source and then successfully to reimplant them in her uterus, is a natural target for these hostile suspicions. This he discovered to his cost last week, when he let it be known that some fertilized ova had not been reimplanted but kept under observation for up to nine days. Mercifully, the British Medical Association has now disowned the advice of one of its committee chairmen that physicians should refuse to collaborate with Dr Edwards. But the rumbling and grumbling continues.

## Ignorance

The fuss is largely born of ignorance, but has a substantial core — the natural (instinctive?) reluctance of all sections of most communities to welcome intervention in human procreation. That this standpoint is in the strict sense irrational does not mean that it is unreal or even discreditable. Church historians must decide to what extent the Roman Catholic interdiction against contraception and abortion stems from these beliefs even though their mainspring is the ethical argument that the premature ending of human life is wrong and therefore sinful. The fact that others than Roman Catholics oppose abortion shows that the ethical argument has general force, as indeed it should have; although ancient Sparta made a virtue of its way of dealing with infants thought to be unfit, for example, modern societies benefit substantially because the legal prohibition of murder has an ethical foundation. The question, for Dr Edwards and the whole research community, is the extent to which such considerations should restrain the pattern of research and its application.

Public alarm about the technique of *in vitro* fertilization, on the development of which Dr Edwards has spent fifteen years of his professional life, is by any test strictly misplaced. Its role in medical practice, while numerically unimportant (there may have been a hundred births or pregnancies so far), offends against neither the ethical nor the religious arguments — unless it should be held that, for the good of their souls, a mutually infertile couple should suffer without complaint the childlessness with which they have been visited. Put simply, the Edwards technique creates life where it would not otherwise exist.

So why should anybody be concerned? Uneasiness that the ova are obtained artificially by a relatively simple procedure is understandable but indefensibly irrational, given the widespread and apparently acceptable practice of artificial insemination with a husband's sperm, and so is not a guide to public policy. The fact that the technique could be a first step towards the use of the uterus of a putative surrogate mother is neither here nor there; even if the male and female donors of sperm and ova sought such a procedure, it is unthinkable that physicians would go along with them — but their professional organizations might helpfully say

so in public. There is a more bizarre possibility that fertilized ova might be deliberately twinned and one partner kept in a deep freeze for serial implantation only when the other had been born and found desirable. This for the time being has not been attempted. Other mammalian embryos have been successfully frozen, thawed out and then reimplanted; other than mechanical stimuli of twinning have not been found. But such techniques will come along. Then, putative parents and their physicians will have to anticipate a complex set of psychological problems for which there are few precedents. They would be well advised to go cautiously, but hardly need to be reminded of that by their professional organizations.

Last week's fuss was, however, provoked by a more immediate issue — Dr Edwards's acknowledgement that he had kept human embryos *in vitro* for up to nine days. Either by accident or from mischief, it seems widely to have been supposed that he had been "experimenting" with them, with all the malign implications that vague word conveys. But in proving a difficult technique such as that on which he and Mr Steptoe are engaged, surely it is not merely legitimate but prudent to learn from the rare opportunities that present themselves what can be learned about such things as the optimum time for transfer to the uterus, or the suitability of various culture media? When such "spare" embryos are disposed of, that is ethically no different from what happens at implantation *in vivo* if an extra ovum fails to take hold.

But Dr Edwards, like many others in the same field, would naturally like to go much further. Last week, he was talking of the possibility that spare embryos might be kept in deep freeze as potential sources of immunological tissues for their congenic siblings, acknowledging that such a use lies far in the future. But there are much more immediate goals for which the use of human embryos will soon be necessary. The extent to which knowledge of what happens — and what goes wrong — in the early stages of natural embryonic growth can be extrapolated from observations of other than human mammals is strictly limited. Sooner rather than later, it will have to be confirmed with human embryos. And one of the next big prizes in biology, an understanding of the process of differentiation, at this stage sought by observations of laboratory animals, will in due course probably require confirmation in human embryos grown beyond the gastrulation stage (two to two-and-a-half weeks). When that time comes, the potential benefits in medicine are also likely to seem overwhelming.

## Committees

The host of committees in Britain now brooding on the ethics of *in vitro* fertilization and what may follow from it must hold this near-certainty firmly in mind. Given the doubts that divide theologians about the time in embryonic development when human life begins (fertilization, implantation, gastrulation — the latest point at which twinning can occur and thus the first point at which one body can have a unique soul — or at some later stage) there is no reason why pointed research proposals should be unnecessarily delayed. But the general reputation of the research profession requires that research projects with human embryos must be well designed, ideally that they should be planned so as to answer questions (test hypotheses) that have already been suggested by animal experiments. Later this month, the Medical



Research Council will be considering a set of criteria drawn up by a committee under Professor Geoffrey Dawes and intended for use in awarding research grants, but which is almost certain to be publicly understood as a kind of code of conduct. Neither the council nor those who may ultimately benefit from a better understanding of early human embryology can afford to be too timorous. The Warnock committee, set up in the summer by the British government (see *Nature* 29 July, p.408) has the wider responsibility of reconciling ethical issues and religious interests with the interests of people working in research. The danger that it will be stampeded by the fuss about Dr Edwards's speech into a flat prohibition of work with human embryos is probably remote. But it is to be hoped that the committee will fight shy of arbitrary distinctions between the permissible and the impermissible. People with sensible questions to ask should be allowed to ask them. Just now, there are not many. Later there will be a host.

## Who pays whom?

*For peace on health service pay, the British government will destroy its universities.*

Mrs Margaret Thatcher's British government has surprisingly taken fright that some radical proposals for reducing the cost to the public purse of certain social services have been leaked to *The Economist*, and is busily denying that it ever intended to take them seriously. With the army of low-paid workers in the National Health Service still disputing the government's offer of salary increases for the current year, it is understandable that the government should not wish simultaneously to fight for the notion that the health service should be financed by insurance payments from those who benefit or who may ultimately benefit. The truth (see *Nature* 23 September, p.287) is that the immediate need is not for a different way of paying for the health services but for a different (and better) way of running them.

One of the adventitious casualties of the disowning by the government of the discussion document on public spending produced by the Central Policy Review Staff (the "think tank") is the reasoned case which it contained for a different way of paying for British universities, one in which British students at British universities would not be paid maintenance grants (means-tested according to parental income) as at present, but would be invited to compete for a large number of scholarships each year. The calculation that it would be electorally dangerous to raise the possibility of financing the health services differently so soon before an election may be accurate, but is it necessary that prudence (not easily distinguished from a lack of courage) should extend so far?

By the end of this week, even Oxbridge will have reassembled for the new academic year, and British universities will be faced with an unprecedented challenge to their pretence of independence — there will be students whom the universities could teach and who are prepared to pay the costs involved but who nevertheless cannot be taken in because the universities have been given quotas of students whom they may educate, and dare not exceed them for fear of incurring financial penalties in years to come. The arrangement, forced on the University Grants Committee by the government, which has failed to find a way of containing the sums of money local authorities are required by existing law to pay to and on behalf of students, is inequitable but also iniquitous. If it persists for long, British universities will be sub-departments of government departments before they know where they are. Should they not rescue Mrs Thatcher from the embarrassment she has been caused by one of her cabinet colleagues' decision to spill the beans about the health services proposals at the wrong time? What, in other words, do the vice-chancellors of British universities have to say about the proposals now in abeyance? They will respond that they are too busy, now that the new term has begun. (In between, of course, they are on vacation.) Briefly, (they say), it is not that they do not have the inclination to fight for the institutions that employ them, but that they cannot spare the time. A pity, history will think.

## Zen and US schools

*"Quality . . . you know what it is, yet you don't know what it is. But that's self-contradictory. But some things are better than others, that is, they have more quality. But when you try to say what quality is, apart from the things that have it, it all goes poof . . . there's nothing to talk about . . . but for all practical purposes it really does exist. What else are the grades based on? Why else would people pay fortunes for some things and throw others on the trash pile?"*

A committee of eminent of US academics with the daunting title of the Conference Board of Associated Research Councils uses this quotation from Robert M. Persig's *Zen and the Art of Motorcycle Maintenance* to express its quandary after taking on the task of rating graduate departments in the United States. The first part of its five-volume study has just been published, covering chemistry, computer science, geoscience, mathematics, physics and statistics/biostatistics programmes. It avoids an overall rating — 1, 2, 3 — for which earlier studies have been criticized. Instead, it attempts a subtle definition worthy of *Zen*'s convoluted introspection. While the study may thus avoid criticism — and be more useful than its predecessors — it may have gone so far in quantifying quality that, in *Zen*'s phrase, "it all goes poof".

The study uses some standard indicators that any professor or grants administrator would agree are useful, such as faculty reputation and the effectiveness of a department in educating students. There is also a pair of interesting new indicators: the number of articles published (normalized for department and faculty size) and "overall influence" of the articles (if they appeared in journals such as *Nature*). Other indicators used tend to correlate with these rankings on reputation, such as programme size and the number of years from a graduate student's enrolment to receipt of a degree. Then there are library size, the proportion of graduate students with federal grants or fellowships, the number of faculty members with federal support and the proportion of graduates with firm job plans after graduation. There are sixteen indicators altogether, but the study group declines to lump them into an overall score for each department. The result is a volume the size of a telephone directory that shows all sixteen indicators for the 596 departments surveyed. Since the reader must decide which indicator to use in determining a ranking, he bears the burden of deciding "what is quality".

The result is an embarrassment of riches for users of the study, who include students deciding where to apply, agencies deciding grant awards and faculty members on the move (not to mention the social scientists who have made a cottage industry out of studying such studies). Predictably, the best departments in the fields covered are the two coasts — Stanford University, the California Institute of Technology and the University of California at Berkeley sweep the board on the West coast (not always in that order), while Massachusetts Institute of Technology and Harvard University are on top in the east. Yet even there, some departments do less well in educating students. Other schools are given ample chance to shine: Cornell is near the top in physics and gets high marks for having greatly improved its geosciences departments in the past five years.

So how seriously will these studies be regarded? By the committee's own admission, there were serious internal arguments about whether it was taking the relativistic approach too far. A dissenting view by three members tellingly criticizes several indicators, especially that which rates departments according to the number of graduates planning to work in institutions that grant PhDs. These days, when so many of the best graduates are going to industry, this measure hardly indicates quality. As a whole, the committee suggested that future studies should also use non-academics as evaluators of departmental worth. Thus, it seems, the values of the committee are in the process of change. This may explain why it found the answer to the question "What is quality?" so elusive.



# US agricultural research criticized

## White House demands more project grants

### Washington

A shake-up in the pattern of agricultural research in the United States may yet materialize. A group of agricultural experts assembled by the White House has urged a substantial expansion of competitive agricultural research grants, now consuming only \$16 million out of the \$1,500 million spent by the US Department of Agriculture (USDA) on research each year. The group says that under the present system, agricultural research is not always of the highest quality or focused on the most critical problems.

Although not new, the group's recommendation may carry more weight than earlier pleas on behalf of USDA's fragile competitive grants programme, for it included representatives of those who have most opposed the programme — the land-grant colleges and the state agricultural experiment stations. Traditionally these have seen competitive funding as a threat to their "formula funds", doled out on a state-by-state scheme dating back to 1887.

Dennis Prager, the assistant director of the White House Office of Science and Technology Policy who organized the group, says that "the agriculture research community has not kept pace with the cutting-edge of basic science". Institutions

difficulty of obtaining funding: "If you're in something that's really medically related, you can get reasonably funded with one or two grants. The problem with the plant business is that you get a little here and a little there. You spend more time writing applications." Or, you go into another field: "It's a good time now for young people trained in molecular biology to go into the plant sciences. But their question is invariably, will I get money to do this?"

Ralph Hardy, director of life sciences at Dupont and a participant in the White House study, points to plant growth regulators as an example of past underinvestment in the plant sciences. Dupont is interested in increasing yields by reducing the wasteful oxygen reaction in plant photorespiratory systems (so increasing the plant's utilization of carbon dioxide, and

thus increasing photosynthesis); but, says Hardy, "the problem is that our base of information has not been adequate" to develop a growth regulator that would accomplish this.

A perennial obstacle to would-be reformers of the agricultural research system is the sensitivities of those within. Despite the pains that the study took to underline the importance of retaining the present formula-funding for the land-grant colleges and state experiment stations. Prager has reportedly been the object of an intense campaign to have him fired.

The recent appointment of Orville Bentley, dean of the agricultural school at the University of Illinois, as the new assistant secretary for science and education at USDA is considered a good sign by those advocating change. Terry Kinney, director of USDA's Agricultural

## Clinch River saved temporarily

### Washington

The fates of two important technical projects, the Landsat satellite system and the Clinch River fast reactor, will now be decided by the lame-duck session of Congress that assembles after the elections on 2 November. That is the effect of the stop-gap spending bill passed hurriedly last week by Congress so as to avoid bringing government business to a halt on 1 October, the beginning of the new financial year. The stop-gap measure, known as a continuing resolution, will allow the Administration to keep on spending at the present rate only until 15 December.

Opponents of the reactor will try again when Congress returns after the elections to deal with the unfinished appropriations bills. They point out that in last week's Senate vote, three senators were absent who are all on record as opposing Clinch River. That would give the opponents a two-vote margin. And in the House, opponents believe that the only thing that stopped them this time was a rule forbidding floor amendments to continuing bills; an amendment to delete funding for Clinch River is thus almost certain to come up in the lame-duck session.

Meanwhile, the Department of Energy (DoE) began site preparation for the reactor after an injunction was lifted by the Federal Court of Appeals in Atlanta. This had been granted by a lower court to environmental groups, which had charged DoE with failing to obtain the proper water permits before beginning construction. DoE appears anxious to make as much progress as possible with the project before another vote comes up in Congress.

The lame-duck Congress will also have the task of straightening out funding for Landsat, which nearly became the victim of budgetary shuffling in last week's con-

tinuing resolution. Operation of Landsat D (launched on 16 July of this year) is due to transfer from the National Aeronautics and Space Administration (NASA) to the National Oceanic and Atmospheric Administration (NOAA) in January 1983. But by late September, Congress had not passed any authorization for the transfer, and the House Appropriations Committee accordingly deleted the \$15 million requested for NOAA's operation of Landsat from the appropriations bill for the 1983 fiscal year.

The House version of the continuing resolution followed suit, holding NOAA's spending for Landsat at the 1982 rate of only \$1.4 million per year. NOAA officials were alarmed; if the new money was not authorized by 15 October, they would not be able to award a contract for operation and maintenance in time to allow for a smooth transition and training of new personnel. The Senate, however, included the higher figure in their version and, luckily for Landsat, prevailed when the two versions were reconciled.

Just before Congress adjourned, a House-Senate conference committee cleared the way for a permanent resolution of the muddle by agreeing on language for a NASA authorization bill which includes the transfer of operating authority over Landsat to NOAA.

The conference committee also agreed to defer a decision on whether to transfer Landsat ultimately to the private sector, a goal of the Reagan Administration. The bill orders the Department of Commerce (of which NOAA is a part) to study alternatives such as a phased transfer; the creation of a corporation along the lines of COMSAT, which operates communications satellites; and keeping the present arrangement.

**Stephen Budiansky**



such as Harvard and Stanford Universities, which are not in the land-grant college system, find it almost impossible to obtain research support from USDA. The establishment of the department's competitive grants programme in 1978 changed that picture only slightly. One consequence, according to Prager, is that "the really exciting basic biological science is going on outside the agricultural system".

Lawrence Bogorad of Harvard University, a plant biologist, argues that while there is expertise within the USDA system, those outside who could contribute something to agriculture are put off by the



Research Service and a member of the study group, says that the competitive grants programme will also be helped by the appointment of a full-time director who will report directly to Bentley. (This plan has provoked concern that the job was going to a career bureaucrat; Kinney says that a career employee will serve on an "interim basis" only until an outside and well-qualified director is chosen.) Directors of the competitive grants programme have previously been on part-time leave from their universities; Kinney says

"one of the reasons that competitive grants have not expanded is that there hasn't been a full-time leader" in a position to lobby for it.

Another participant in the White House study, Lowell Lewis, director of the University of California's agricultural experiment stations, states another reason why the time may be ripe for a boost in competitive funding is the mood of Congress and the Administration. "I think we've heard the message clearly that we're not going to get across-the-board increases

in formula funds", he says; a more focused programme — such as competitive grants for basic research — is much more likely to be viewed with favour. Even allowing for the importance of formula funds to those in the land-grant system (and Lewis, like many others, vehemently defends the formula funds as essential to the research that every state needs to conduct on local agricultural problems), these budgetary facts may help to soften resistance to an expanded competitive programme.

Stephen Budiansky

## Plutonium reprocessing

# Fast breeder development

Spent fast breeder fuel — a mixture of plutonium and uranium oxides and a variety of active fission products — has been reprocessed into new fuel elements at Dounreay, Scotland, with 99.6 per cent efficiency, the United Kingdom Atomic Energy Authority announced last week. The efficiency (the ratio of plutonium leaving fuel fabrication to the plutonium introduced to the reprocessing plant) is crucial to the operation of a fast breeder fuel cycle and — until the Dounreay result — it had been expected to be only 96 per cent at best. Thus Dounreay has reduced potential plutonium fuel cycle losses from an expected 4 per cent to 0.4 per cent.

The figure is not only important for fast breeders in general — it might also be expected to increase Britain's chances of arranging international collaboration (and cost sharing) in the next stage of fast breeder development. France, for example, is leading the world in the construction of the first commercial scale (1,200 MWe) breeder, Superphénix (which should go critical in mid-1984 and feed power to the grid by the end of that year). But France is at least three years behind the United Kingdom in fast fuel reprocessing technology.

Could Britain use its reprocessing success as a bargaining card? Sources at the Commissariat à l'Energie Atomique (CEA) think not. According to CEA, 90 per cent of the Superphénix core already comes through reprocessing the fuel of Phénix, its 250 MWe predecessor. But this has been achieved through a patchwork of inefficient processes both at La Hague (the commercial reprocessing centre near Cherbourg) and at Marcoule, a CEA research and production centre on the Rhône.

The largest French fast fuel reprocessing plant — at Marcoule — can handle two tonnes of spent fuel a year, only a quarter of the amount that can be handled at Dounreay. Moreover, efficiency at Marcoule is certainly lower than at Dounreay, although CEA sources express themselves "satisfied", allowing for the fact that the plant is old.

Marcoule is being upgraded, and will be able to handle 5–6 tonnes of fast fuel a year by 1984. But this in turn is only one quarter

of the expected reprocessing needs of Superphénix. Excess spent Superphénix fuel is to be stored in cooling ponds, awaiting the construction of a full scale commercial fast fuel reprocessing plant. To be economic, according to CEA plans, this plant would have to handle the output of up to eight breeders of the size of Superphénix, and have a throughput of up to 170 tonnes a year — a more than twentyfold scale-up of even the Dounreay plant. But, so far, French chemical reprocessing technology has been poor — La Hague, for example, has been working well below design capacity because of clogging and other problems.

French sources say, however, that the La Hague hold-ups have been "good experience", and that new plants — such as the upgraded Marcoule — will function well. A light water fuel reprocessing plant built by the French at Tokaimura in Japan is said to be operating efficiently.

Thus the French do not see the Dounreay success as an entry ticket to French reactor technology — particularly as Phénix appears, on the face of it, to have been such a success in comparison with the Dounreay prototype fast reactor (PFR). The two reactors are of the same scale (250 MWe) and vintage (1973). In recent years, PFR has shown a load factor, or proportion of electrical power output to the maximum theoretically possible, of only 20 per cent (in fact just 14 per cent in 1981–82). (By comparison, the world average for pressurized water (thermal) power reactors is about 60 per cent.) But Phénix has recently shown a load factor of around 70–80 per cent.

According to officials at Dounreay, the difference is that the Phénix stainless steel sodium-water heat exchangers, which are subject to leaks, are modular and can be changed quickly. But the cost of such changes would be uneconomic for a commercial reactor. PFR does not have modular exchangers, and so is out of action longer when a leak occurs. Superphénix, however, as a prototype commercial reactor, does not have modular exchangers, and this is leading to some fluttering hearts in France, say Dounreay scientists.

Robert Walgate

## Blow for EISCAT

The European Incoherent Scatter Facility (EISCAT), opened with much fanfare in August 1981 to probe the Arctic ionosphere, is off the air: a valve has blown. But this valve, a klystron, is 4 metres long and costs \$200,000.

Luckily, the EISCAT planners had a spare, now being put in place. According to EISCAT director Dr Murray Baron, speaking from Tromsø, Norway, the cause of the original valve failure will not be known until it has been disassembled by its manufacturers, Varian Associates of California. He does not blame design error, although such problems led to a three-year delay in the start-up of the facility.

Varian had guaranteed the klystron for 1,000 hours of operation or a year of service; this valve had operated for 3,000 hours when it failed at the end of August, and Baron had expected another couple of thousand hours from it. Although the EISCAT committee has set aside a contingency fund to buy new or reconditioned klystrons every two or three years, this failure after just over a year would cause financial problems if it were repeated. Besides which if the spare valve were to fail, it would be seven to nine months before Varian could produce another, leaving EISCAT without transmitters and thus data. EISCAT has two transmitting systems, one in the UHF (933 MHz), whose klystron has failed, and one in the VHF (224 MHz) which is not yet delivered. The fear is that the fault may be in the equipment, not the valve, in which case the spare would also blow.

Otherwise, EISCAT seems to have been a success. Its three-week summer campaign this year involved rocket flights and television observations in parallel with EISCAT measurements and is said to have given interesting data on aurorae, sporadic E-layers and low-ionospheric spectra.

EISCAT is an international collaboration between West Germany, France, the United Kingdom (each contributing 25 per cent of capital and running costs), Sweden and Norway (10 per cent) and Finland (5 per cent). The original capital cost was £13 million.

Robert Walgate

## US national security leakages

# Academics mostly absolved

Washington

US universities have not done significant harm to US national security by the leakage of technical information. This is the conclusion of a report\* by a panel of the US National Academy of Sciences (NAS) released last week. While agreeing with the government that the leakage of information is a "substantial and serious" problem, the panel says it could find no "specific evidence" in the secret material to which it had access that the academic community is responsible, for which reason controls should be very limited.

However, the members of the panel avoided direct criticism of the Reagan Administration, perhaps hoping that it will follow suggestions made in the report in balancing national security and scientific needs. The panel's findings have already been presented to Mr Caspar W. Weinberger, the Secretary of Defense, Mr Joseph Clark, the President's National Security Advisor and Dr George A. Keyworth, the President's Science Advisor.

The problem first arose in 1978 when the US intelligence community attempted to censor some mathematics papers having applications to cryptography. The most recent incident involved the forced withdrawal, by the Department of Defense, of more than 100 papers from a San Diego photo-optical society meeting. Two weeks ago, President Reagan said that if his Administration had gone "too far" in any particular case, "we will rectify that" (*Nature* 23 September, p.289, and 30 September p.383).

Although disputing the Administration's view on some points, the panel confirms that Soviet intelligence is trying harder to obtain sensitive information in fast-moving fields, such as electronics and software, from US universities, which it finds more vulnerable than US industry.

The panel, under the chairmanship of Dr Dale R. Corson, president emeritus of Cornell University, concluded that the two laws now invoked to dissuade scientists from publishing or presenting their work — the Export in Arms Regulations and the International Traffic in Arms Regulations — were unsuitable for controlling the scientific community. Corson said that they were developed for controlling the export of equipment. They are inappropriate for scientific work, he said, in which "the network of communication is so basic and so broad", in which people travel all over the world talk to each other, and give each other preprints, in which "graduate students talk to everybody." Instead, the committee recommended simple, practical and well understood control procedures that would not ordin-

arily inhibit scientists' rights of communication and publication.

As for the San Diego meeting, the panel found the government's procedures inappropriate, Corson said, although he would not comment on whether the papers should have been withdrawn.

The proper method of restricting information is the classification system, Corson explained, but it should be used only:

- if the technology is rapidly unfolding from basic research to applications;
- if it has direct military application;
- If it could give the Soviet Union a near-term military advantage; and
- if the United States is the only source of the information "or other friendly nations that could be the source have control systems as secure as ours".

The panel called on both the Department of Defense and the Office of Science and Technology Policy under Dr Keyworth to play a key role in formulating policy on scientific censorship. It also agreed with the

Administration's view that the net flow of information in US-Soviet exchanges favours the Soviet Union. But Corson warned that the United States received a great deal in return, including the basic concept of tokamak fusion reactors.

The report notes that the only documented abuses of open university research "almost always involved episodes in which visitor status was abused by Eastern bloc scientists". A visitor would study fields beyond the agreed area of study, or spend time in the library, apparently looking up information asked for in advance by Soviet intelligence and unrelated to the ostensible purpose of the visit.

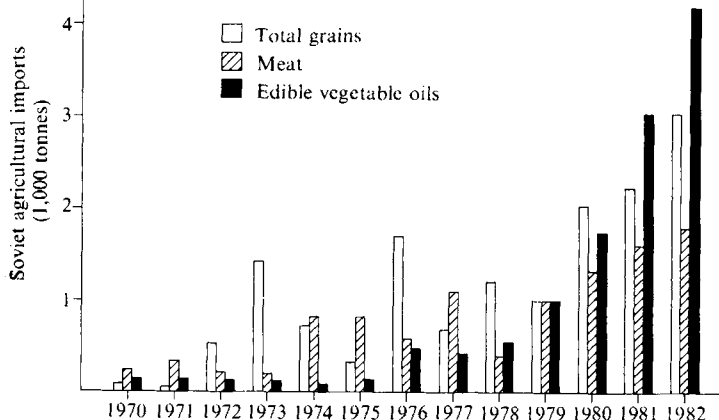
"A significant fraction of all Soviet scientific visitors are believed to have intelligence roles" the report says. "One should assume that almost all Soviet technical visitors to the United States are prebriefed about specific acquisition needs, and it is certain that Soviet visitors to other countries are required to report on their foreign experiences. There is evidence that the quality of their reports is a possible factor in decisions about their future travel applications".

Deborah Shapley

## Soviet dependence on food imports

The Soviet Union's rapidly growing dependence on imports of food over the past four years reflects a serious deterioration in the Soviet agricultural system according to a study, *US and Soviet Agriculture: the Shifting Balance of Power*, by the Washington-based Worldwatch Institute. What had been a pattern of imports in bad years only (with production failure blamed on bad weather) has given way to a sustained and increasing reliance on imports.

Since 1978, Soviet production of grains, meat, milk, vegetables and sugar has actually declined, following decades of erratic but significant gains. The report blames the inability of central planning to keep up with the complex and shifting demands of modern, high-yield agriculture for machinery, fertilizer and chemicals. "The evolution of insect resistance shows little respect for the time-lags of Five-Year Plans." A neglect of marketing facilities is also partly to blame.



Soviet dependence on imports is mirrored by growing US dependence on export markets. Since 1972, exports of grain have grown from approximately 40 million tonnes per year to over 110 million. (The other chief grain exporters, Canada, Australia, Argentina and France, each export less than 25 million tonnes a year.)

Although less than 20 million tonnes of this year's US exports will go to the Soviet Union, the study says that without the increases in US exports in the past decade "there would not be nearly enough grain to meet all world import demands at current prices; and certainly not enough to support the growth in Soviet imports".

The report also suggests that Soviet reserves of hard currency may be the factor that limits Soviet food imports. And that, said Lester Brown, the author of the study, means that "US farmers have a greater stake in getting the [Soviet — Western Europe natural gas] pipeline built than almost anyone else".

Stephen Budiansky

\*Scientific Communication and National Security, (National Academy Press, Washington DC, 1982).

## Renewable energy

# Europe's gloom

**Brussels**

As preparations begin to launch a new programme for EEC research into the use of renewable energy sources, figures released by the International Energy Agency (IEA) in Paris reveal a slackening in the efforts of the agency's 21 members to develop the potential of solar, biomass and other alternative energy sources. According to IEA, the countries of the industrialized world have reduced research spending from a peak of \$1,102 million in 1980 to \$1,076 million last year, although there is no accompanying decline in the field of nuclear research, where spending rose by 4.2 per cent between 1980 and 1981 to a total of \$4,500 million.

In spite of the encouraging trends in the pricing and supply of oil on world markets, reports from both the European Parliament and the European Commission emphasize that there should be no relaxation of efforts to make EEC less dependent on imported energy. EEC's vulnerability to political pressures shows no signs of decreasing, as is shown by the controversy over the Siberian gas pipeline, although the prospects of a major increase in the price of oil has for the time being receded.

Experts at the European Commission are worried that the complacency revealed by the IEA figures will cause a lack of enthusiasm to invest seriously in renewable energy sources. As EEC's own projects demonstrate, the time is now ripe to make the transition from the laboratory to commercially viable investments. The European Commission is now putting forward a proposal to stimulate energy investment with a financial aid package totalling £600 million over the next five years in areas most likely to be neglected — renewables, district heating systems and converting oil-fired plants to coal. The money would be used to meet the cost of interest rebates on loans.

The research sponsored by EEC has been reviewed in detail in a number of reports produced for a debate held last month in the European Parliament. There are three separate programmes — one on solar energy at the Joint Research Centres costing \$25.5 million, a series of demonstration projects budgeted at \$22.5 million and run by the Energy Directorate General, and a joint action programme worth \$45 million run by DG XII (Directorate General for Research, Science and Education). These programmes are likely to be brought under one roof in the third research and development programme beginning in 1983.

The new programme is likely to continue to invest most heavily in the areas which have received most support in the past, but perhaps in different proportions. Spending so far shows a strong bias towards solar

energy, with 34 million ECUs (European Currency Units) (£60 million) going on photovoltaic cells, 43 million ECUs on solar panels and other thermal applications and the remainder split between biomass (24 million ECUs), thermodynamic power plants (12 million ECUs) and wind energy (1 million ECUs).

Biomass is likely to receive greater attention in the next programme — the Commission calculates that if it is possible to provide 17 per cent of the Community's total energy needs from renewables, as much as 8 per cent could come from biomass, half from existing wastes and half from crops grown for energy use. A detailed report produced for the European Parliament on the future of biomass by Madron Seligman (UK, Conservative) stresses that there is a need to get demonstration projects under way and to assess which source of biomass would be the most promising in which regions or agricultural systems and with which technology.

He points out that there is a constantly widening choice of research techniques using an expanding range of biomass sources including sugar, artichokes, maize, vegetable oils, trees, reeds and agricultural wastes. Only a few sources will be suitable for large-scale industrial exploitation, but many others could be viable on a small scale. The European Parliament's resolution calls on member states to provide 130 million ECUs for a five-year biomass programme starting in 1983. This would include 20 million ECUs for agricultural grants which would be used to encourage farmers to switch from producing agricultural surpluses to produce energy crops, or to grow energy crops as well as their normal crops.

Seligman points out that subsidies are already being used to support surplus production of sugar beet and other crops and would be better applied to helping EEC to become more self-sufficient in energy. They could even be used to persuade farmers in Thailand, for example, to convert their manioc into gasohol and thereby reduce pressure on Community-produced cereals and also reduce their own energy imports.

**Jasper Becker**

**Bioriches**

## Banker's hope

Take biotechnology, \$50 million and a Rothschild — even the one who knows what biotechnology is all about — and surely you have a recipe for a goldmine: that seems to be the calculation that inspired the formation of Biotechnology Investments Limited a year ago. The company, of which Lord Rothschild is chairman, is an offshoot of N.M. Rothschild Asset Management Limited, which is in turn the venture capital part of the merchant bank called N.M. Rothschild & Sons Limited, the host for the twice-daily meeting of the cartel that fixes the price of

gold on the London metal market, whose chairman (until he recently became its president) was the biological Rothschild.

The calculation appears to have been good, and may yet prove accurate. The company's first annual report, now published, promises a dividend of 10 cents for each nominally 2 cent share (for each of which investors will have paid \$10.00), a yield in the first year of rather less than 1 per cent. And that has been made possible only because Biotechnology Investments earned the best part of \$2 million by investing in the money markets. The \$16 million invested in biotechnology companies at the end of May produced only \$38,000 in the financial year. But it seems to be agreed that this will be a long haul.

Lord Rothschild, distinguished for his work on the fertilization of mammalian ova and best known for his reorganization of British public spending on civil science in 1971, when head of the government's Central Policy Review Staff ("think tank") is now interestingly to be observed in that nexus where his instincts as scientist and banker are nicely balanced. He has a small team of bright people to assess investment proposals (and with whom to gossip), but a banker's unwillingness to take too many risks.

One consequence is that, so far, venture capital has been invested only in US companies, including \$250,000 in Advanced Mineral Technologies (founded by Dr Corale Brierly in New Mexico), £1.17 million in Agrigenetics (David Padwa) and \$2.01 million in Repligen (founded by Drs Alex Rich and Paul Schimmel of MIT). The company is a little embarrassed that it has not yet backed a British venture, and has become an eloquent source of opinion about the dearth of entrepreneurship in Britain. (Briefly, tax rates are less important than attitudes.)

The company's annual report is a useful banker's view of how this handful of private companies is making headway. Only one unquoted investment, in the Seattle-based monoclonal antibody company Genetics Systems, has been written down (and then only by 2 per cent); that in Agrigenetics is now valued at \$1.92 million.

The investment fund's strategy, spelled out in the annual report, is to invest primarily in new and start-up companies, where it is acknowledged that the risks but also the rewards are potentially higher than in the public companies whose shares have cost Rothschild \$8 million (and which include Amersham International). The objective is to find companies with "scientists of high calibre" but also first-rate business managers. The Rothschild bank will be rewarded on a kind of deferred payment plan, collecting a fifth of the increased value of investments in new companies only after five years, and if the fund's shares are then worth more than \$15.00 each.



## Swedish science medal

## One way out

## Stockholm

Last week, Sweden's new prize for science, endowed by the late Holger Crafoord, inventor of the blood dialysis machine, and his wife Anna-Greta Crafoord, was presented for the first time. The prize was awarded jointly to two mathematicians, Professor Louis Nirenberg of the Courant Institute of Mathematics (New York University) and Professor Vladimir Igorevich Arnold of the State University of Moscow. Only one of the winners, however, was present in Stockholm to receive the prize medal from King Carl Gustaf. Professor Arnold had been unable to obtain an exit visa.

The Crafoord prize is already widely regarded in Sweden as a "mini-Nobel", although Dr Crafoord (who died earlier this year) always found the comparison irritating. There are several significant differences between the two prize funds —

in particular, the Crafoord prize will be awarded for one subject only each year — this year it was the theory and application of non-linear differential equations — rotating between mathematics, astronomy, the geosciences and the life sciences. Nevertheless, the value of the prize money — 350,000 Swedish crowns (£35,000) this year — is at least comparable with the Nobel prizes (which are now around £100,000). The fact that, like the Nobel prizes in physics and chemistry, the Crafoord prize winners are selected by the Royal Swedish Academy of Sciences, reinforces the association. The Swedish media, therefore, when Professor Arnold failed to arrive, inevitably compared his absence to that of the three "missing" Nobel laureates of recent years — Boris Pasternak (1958), Aleksandr Solzhenitsyn (1970) and Andrei Sakharov (1975). In fact the cases are not entirely comparable. The three Nobel winners — two for literature and Sakharov for peace — received their awards for writings and actions which the Soviet authorities considered hostile. Arnold's absence, although explained by many of his colleagues attending the prize

## Australian physiology congress

## ICSU blacklist

## Canberra

The Australian Academy of Science has averted the withdrawal by the International Union of Physiological Science (IUPS) of sponsorship for the physiology congress scheduled to take place in Sydney in August–September next year. As a consequence of the Australian government's refusal to grant visas to two Soviet scientists who were to attend the biochemistry congress held by the International Union of Biochemistry (IUB) in Perth last month (see *Nature* 9 September, p.97), the International Council of Scientific Unions (ICSU), of which both IUB and IUPS are constituent bodies, proposed to blacklist Australia as a conference centre.

Bowing to pressure from the president of the academy, Professor Arthur Birch, and spurred by the impending crisis, the Australian government has now publicly stated what it had previously implied — that it is willing to bend the rules a little to allow Soviet scientists who are also senior officials to attend conferences in Australia, provided sufficient notice is given. This is a softening of its blanket banning of all high-ranking Soviet nationals regardless of their purpose in seeking entry into the country. On 31 August, the minister for foreign affairs, Mr Anthony Street, wrote to Professor W.N. Christiansen, the foreign secretary of the academy, to say "the government's procedures in relation to the entry of senior Soviet nationals wishing to participate in genuinely international conferences are not inflexible". On 9 September, he wrote: "In cases involving attendance at multilateral conferences, [the government] will weigh [its policy] in seeking to ensure the entry of all *bona fide* participants provided, of course, normal entry criteria are met. In this respect the government will maintain conditions similar to those applied by like-minded Western countries in their national interest." On 9 September, Mr Street said in the House of Representatives: "The Soviet Union has clearly embarked on preventing Australia being used as a venue for international conferences. We are not prepared to give the Soviet Union that satisfaction."

By spelling out its policy, the government enabled the academy to reinstate its assurance to IUPS on free circulation while still maintaining its sanctions against the Soviet Union for its invasion of Afghanistan. In addition, however, IUPS asked the government to decide on visa applications no later than 30 days before the physiological congress. The minister agreed, but pointed out that the ICSU rule that visa applications should be made 90 days in advance should apply to Australia in order to give the government as much time as possible to consider applications.

Vimala Sarma

## Biotechnology drain

Like a man trying to fill a bathtub while not knowing whether the plug works, the British Science and Engineering Research Council (SERC) last week asked the Institute of Manpower Studies at the University of Sussex to look into the rate of emigration among qualified British biotechnologists. The council has apparently taken to heart the announced departure of Professor D.C. Burke of the University of Warwick, who is to become research director of a Canadian company.

The project, called a "partial survey" will concentrate on the outgoing flow of people. To begin with, the Sussex institute will attempt a definition of which kind of people are considered "key" by their contemporaries. Thereafter, it will use the scientific network to compile a list of those who have left the United Kingdom in the past two years, and of their destinations.

A spokesman for the institute said this week that he had no idea how many people were at risk of being seduced away from Britain, but that he imagined that the institute would be able to identify a few hundred people in this category. Graduate students would be included where appropriate. The spokesman said that the institute had insisted, in its dealings with SERC, on a guarantee of confidentiality: numbers might be disclosed, but not names and addresses.

Even so, it seems to be part of the intention that there should in future be a means by which British research councils should be able to "keep in touch" with departed biotechnologists, reminding them "when the time is right" of the benefits of returning. The custody of the register remains to be decided.



ceremony and symposium as due to his having signed a protest letter in defence of a colleague some years ago, was not directly related to the research for which he received his prize. Moreover, Solzhenitsyn and Sakharov were not so much prevented from leaving the Soviet Union to collect their prizes as afraid that, if they did leave, they would not be allowed to return.

An even more significant difference, perhaps, was the attitude of the Soviet embassy in Stockholm. In the cases of the three "absent" Nobel laureates, the Soviets boycotted the ceremony completely. At the Crafoord ceremony, however, the embassy was represented by its scientific attaché, who clearly found the situation embarrassing. But he had come equipped with a cutting from *Izvestiya*, listing the results of the election of new foreign members to the Soviet Academy of Scientists. These included three Swedes: Lennart Carlsson, Jerkar Porath and Lars Ernster; three Americans: Russell Bardin, Emil Smith and Gilbert White; and two Britons: John Bertrand Adams and Lord Todd. This, he said, showed the Soviet desire for continuing fruitful international cooperation in science.

Vera Rich

# CORRESPONDENCE

## Malaria resurgence

SIR — We are puzzled that our research on malaria resurgence has apparently been misinterpreted and would like to take the opportunity provided by Drs Sharma and Mehrotra to clarify our argument<sup>1</sup>.

Most of their evidence has very little bearing on our case. In southern and central India, widespread anopheline resistance to common pesticides developed after 1970, not during the early and mid-1960s as they seem to suggest (see table). It is also clear that agriculture, not mosquito eradication, is the primary source of pesticide use in these regions.

Comparing annual parasite index figures with agricultural data for the period 1970–76, for example, it is apparent that human infection rates rose most dramatically in districts where local farmers — not public health officials — applied the greatest quantities of insecticides<sup>2</sup>. Moreover, as Sharma and Mehrotra themselves admit, “accurate figures for DDT used in agriculture (1960–1972) are not available” — a major weakness in their argument that “use of DDT in agriculture was far less than for public health”.

On the contrary, officials in FAO and the US Department of Agriculture concur that such figures grossly underestimate the amount of DDT and other chemicals applied by Indian farmers. More important, perhaps, such inaccuracies reflect a significant lapse in reporting during the critical years (1966–72), when high-yielding varieties of rice (needing heavy insecticide use) were introduced into the region. And finally, whereas irrigation schemes may have contributed to the proliferation of mosquitoes, the fact that these mosquitoes quickly became resistant to common pesticides can best be explained by the ways in which farmers used such compounds in newly-irrigated fields.

Thus Sharma and Mehrotra speak more cogently to a different point, namely, that public health authorities in India applied startling quantities of DDT in areas where this compound had long since ceased to be effective against *Anopheles* vectors. The results of this procedure may be even more serious than they recognize: according to the World Health Organization (WHO), resistance to DDT and related chemicals now affects five (not two) of the seven anopheline species that transmit malaria<sup>3</sup>.

Similarly, on the subject of malathion, Sharma and Mehrotra suggest that malaria vectors remain susceptible to this compound in regions where it has not been used for mosquito control but primarily for agricultural purposes. And yet, according to WHO, “*A. culicifacies* . . . is resistant to

DDT, HCH and malathion in 114, 33 and 2 districts (one each in Maharashtra and Gujarat) respectively, covering a population of more than 250 million. Resistance to malathion has been detected in other areas — one district in Andhra Pradesh and another in Haryana — where the compound is being used for the control of agricultural pests and not in any public health programmes<sup>4,5</sup>”.

Finally, we should add that, unlike Sharma and Mehrotra, we do not possess great faith in the possibility that *Anopheles* vectors will regain their sensitivity to DDT or other chemicals when these have been withdrawn from the environment for some period of time. Although such events may take place on a limited scale, the weight of entomological evidence speaks for continued or heightened resistance, not “reversion”<sup>6</sup>. Such considerations are surely the reason WHO has begun to pour its slender resources into studies of vector biology and the development of novel insecticides.

As we mentioned in our article<sup>15</sup>, this quest may also be fruitless: even if these compounds manage to circumvent the largely unknown mechanisms whereby *Anopheles* mosquitoes detoxify their assailants, they are likely to be so expensive that few governments will be able to afford them. At that point, perhaps, health authorities will give serious attention to what a number of entomologists and other specialists have been saying for some time: that we must develop a rational and comprehensive strategy of ecosystem management which takes into account the need to control both human disease transmission and agricultural pests. These are not separate problems, and the current manner of solving them — indiscriminate and self-defeating application of pesticides — will hopefully be assigned a place in our medical history similar to the chapter which now describes how our predecessors removed evil humours from the blood.

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### Recent reports of *Anopheles* resistance to commonly used pesticides in India

| Date of research | Compound                            | Vector                   | Place      | Ref. |
|------------------|-------------------------------------|--------------------------|------------|------|
| 1960 ff.         | DDT, HCH                            | <i>A. culicifacies</i>   | Gujarat    | 6    |
| 1973             | Malathion                           | <i>A. culicifacies</i>   | Gujarat    | 7    |
| 1973–78          | DDT, HCH                            | <i>A. culicifacies</i>   | Karnataka  | 8    |
| 1973–78          | DDT, HCH                            | <i>A. fluviatilis</i>    | Karnataka  | 9    |
| 1976             | DDT, HCH                            | <i>A. stephensi</i>      | Tamil Nadu | 10   |
| 1976–77          | Propoxur                            | <i>A. stephensi</i>      | Tamil Nadu | 11   |
| 1978 ff.         | DDT                                 | <i>A. culicifacies</i>   | Tamil Nadu | 12   |
| ND (post-1976)   | DDT, HCH                            | <i>A. subpictus</i>      | Tamil Nadu | 13   |
| ND (pre-1980)    | Fenitrothion                        | <i>A. culicifacies</i>   | Not spec.  | 14   |
| ND (pre-1980)    | DDT                                 | <i>A. philippinensis</i> | Not spec.  | 14   |
| ND (pre-1980)    | Malathion, Fenitrothion, Jodfenphos | <i>A. stephensi</i>      | Not spec.  | 14   |

## Sequences on-line

SIR — The recent letter from C. Saccone *et al.* (*Nature* 1 July, p.8) implies that nucleic acid databases are available for European research workers only by mailing of magnetic tapes. Your readers may be interested to know that an extensive nucleic acid sequence database, including the one released by the European Molecular Biology Organization (*Nature* 15 April, p.596) as well as one developed and maintained by the Los Alamos Scientific Laboratories, can be searched and analysed by direct on-line connection to a Digital Equipment Corporation 2060 computer in Paris, France. This service is provided and maintained by IntelliGenetics, Inc.

Over thirty research groups in six European countries use this service. The databases are in a format compatible with the fastest database search algorithm and the most sophisticated sequence analysis and alignment programs.

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## Preventing wars

SIR — The recent letter from the Medical Association for Prevention of War (MAPW) (*Nature* 9 September, p.102) contains proposals which confuse what international bans and agreements on nuclear weapons can and should do, and what they cannot and should not try to do. I say “contains” because it starts off by suggesting proposals which are reasonable extensions of existing principles with which I have no disagreement. These, however, are used to imply the legitimacy of further ideas of a quite different nature.

The prevention of wars is concerned with many antecedents, some of them being remote from the salient causes of medical injury. The causes of war are separate from the causes of the effects they have on those involved in them. The cause of the growth of an individual tree in a wood is a different thing from the causes of the processes which affect the wood as a whole which may include human economics. MAPW confuses them, mistaking as the same thing the causes of personal loss and the causes of the conflict in which that loss occurs. To remove the latter will remove the former, and is done frequently by the United Nations and the United States. However, the removal of the former has never led to the removal of the latter.

The main antecedent to war is always the expectation that initiating a military action will lead to gains outweighing the losses incurred in making it. No sane extension of prohibition treaties can be made which allows greater expectation of gain from military action. But this in effect is what MAPW proposes. It is far better to live in a world where there are vast known and undoubted negative expectations of gains from initiating nuclear war but which contains many times the number of warheads potentially to extinguish the human species than to live in one where there are insufficient warheads for that but for which there is a real worthwhile gain for one party to initiate their use.

J.R. SKOYLES

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## NEWS AND VIEWS

# Connections between the nervous, haematopoietic and germ-cell systems

from Edward S. Golub

SIMILARITY or congruence between the immune and nervous systems has been the topic of philosophical conversation between immunologists and neurobiologists for a very long time because superficially the two systems seem to have faced and solved some of the same problems. Both have the primary function of recognizing and reacting to the outside world; both have a remarkable degree of diversity which allows them to have an enormous range of responses; and both learn, remember and even forget. This connection has been made even more intriguing because of the growing evidence that emotions have an effect on susceptibility and resistance to disease<sup>1</sup>.

The notion of the connectedness of the two systems was strengthened a decade or so ago by two separate lines of evidence. In the first, it was shown that the brain shared an antigen with thymus-derived lymphocytes (T cells)<sup>2</sup>. This observation was extended to show that the antigenic cross-reactivity was even greater, extending, in fact, to the whole haematopoietic system<sup>3</sup> (from which the immune system is derived), including the pluripotent stem cell, pre-T cell, B cell, erythrocyte and granulocyte. Very recently an antigen on T suppressor cells was shown to cross-react with cultured oligodendrocytes<sup>4</sup>.

The second line of evidence showed that in rats, hypothalamic lesions had a profound suppressive effect on the immune response<sup>5</sup>. It could be argued that the hypothalamus is such a pivotal gland, producing oxytocin, vasopressin and pituitary hormone-releasing factors, that the effect on the immune response was non-specific. However, studies done in the opposite direction<sup>6</sup> showed that animals undergoing an immune response exhibit an increase in the firing rate of individual neurones in the ventromedial nucleus of the hypothalamus.

These results all argued for some kind of functional connection between the nervous and immune systems. Furthermore, as the

hypothalamus makes neurosecretory factors which control hormonal release from the pituitary, it might not be surprising if neuroendocrines released by the pituitary — ACTH and endorphins — had an effect on the immune response. Two recent papers show just this connection.

The first paper<sup>7</sup> shows that ACTH and  $\alpha$ -endorphin (but not  $\beta$ -endorphin) are capable of suppressing the ability of cultures of mouse spleen cells to generate antibody responses to sheep red blood cells (SRBCs) and dinitrophenol (DNP)-Ficoll. SRBC is a thymus-dependent antigen and DNP-Ficoll is thymus independent, so the regulatory action of the hormones might be at the B-cell level, although action at the level of the macrophage or suppressor T cell is also possible.

The second paper<sup>8</sup> shows that addition of  $\beta$ -endorphin (but not  $\alpha$ -endorphin) to cultures of rat spleen lymphocytes causes a marked, but highly variable, increase in the proliferative response of these cells to the T-cell mitogen concanavalin A.

Taken together, these two papers strongly suggest that the neurohormones can function as regulators of the immune response. It is not yet known whether they are acting on B cells, helper and/or suppressor T cells or macrophages but the differences between the two studies almost certainly point to the fact that many types of cells in the immune network may be susceptible to their action, that is, express receptors for neurohormones.

Several fascinating questions are raised. In one of the studies,  $\alpha$ - but not  $\beta$ -endorphin was active, yet  $\beta$  and not  $\alpha$  was active in the other. In the first case, the effect could be inhibited by naloxone, a competitor for opiate receptors, whereas in the second naloxone had no effect. Thus it seems clear that the cells of the immune system may have several types of neurohormone receptors. It would be of great interest to know whether the antigens shared by the nervous and haematopoietic systems are in fact receptors for these neurohormones.

Another group of cells, the primordial germ cells, have met some of the same problems as the haematopoietic and

nervous systems. These cells arise next to nervous tissue in the embryo and, like both the haematopoietic and nervous systems, they retain great differentiative potential and express that potential after extensive migration. Moreover, several defective haematopoietic mutants in mice have concomitant defects in germ cells (*W*, *SI*, *bg*) and it is known that some antigens are shared by nerve cells and germ cells<sup>9</sup>. In amphibian fate maps, germ cells and haematopoietic cells arise near each other and in hydra, the interstitial cell gives rise to both nerve and germ cells<sup>10</sup>. Since we already know that there is extensive antigenic cross-reactivity between nervous and haematopoietic tissue, it will be interesting to see whether antibodies directed against antigens on either one of these cells will also react with germ cells. This possibility is made more intriguing by the recent report<sup>11</sup> that ACTH- and  $\beta$ -endorphin-related peptides are present in multiple sites in the reproductive tract of male rats. The peptides are of local, rather than pituitary, origin.

Assuming the connections between these three systems are real, one can speculate about the functional reasons. Did they share common ancestral cells and are the antigens and receptors vestigial? Do they have common types of problems to solve and so have evolved the same mechanisms? Or, perhaps most interesting from the point of view of the organization of cells and possible clinical manipulations, have they evolved a regulatory and functional interdependence which we are just beginning to see? □

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# Brain catecholamines in schizophrenia — a good case for noradrenaline

from Oleh Hornykiewicz

In the last two decades, brain catecholamines, and in particular dopamine, have played a prominent part in hypotheses of the neurobiology of schizophrenia. Briefly, the dopamine hypothesis states that in the schizophrenic brain, some critical dopamine systems are in a state of continuous overactivity or supersensitivity. The hypothesis rests on the following evidence<sup>1-3</sup>: (1) neuroleptic drugs that are beneficial in treatment of many schizophrenic patients have strong brain dopamine receptor-blocking properties; (2) the antipsychotic potency of neuroleptics correlates well with their dopamine receptor-blocking activity as determined in acute experiments or *in vitro*; (3) drugs which, *inter alia*, increase brain dopamine activity (amphetamine, L-dopa) worsen schizophrenic symptoms; (4) an increased number of neuroleptic binding sites representing the D-2 type of dopamine receptor has been detected in the schizophrenic brain, specifically in the nuclei of the basal ganglia, that is, the caudate nucleus, putamen and nucleus accumbens.

Given this evidence one might ask whether any other hypothesis is necessary. At first sight, the dopamine hypothesis appears to explain satisfactorily many clinical observations and it has undoubtedly been of great heuristic value in predicting the antipsychotic activity of new compounds. The hypothesis is not, however, free from difficulties.

The main body of support for the dopamine hypothesis comes from the 'neuroleptic evidence'. However, as a rule, hypotheses based on drug effects have to be regarded with caution. That drugs suppressing brain dopamine activity (that is, neuroleptics) improve schizophrenic symptoms, and drugs stimulating dopamine activity (for example, amphetamine, L-dopa) worsen these symptoms, is reminiscent of the effects of cholinergics and anticholinergics in Parkinson's disease. Thus, not so long ago, all known drugs for Parkinson's disease were anti-acetylcholinergic and a satisfactory correlation existed between their anticholinergic potency and their clinical efficacy<sup>4</sup>. In contrast, cholinergic drugs were known to worsen Parkinsonian symptoms<sup>5</sup>. From these observations one could very easily have concluded that Parkinson's disease is a disease of the cholinergic system. In fact, Parkinson's disease is not a disease of the cholinergic system, but is due primarily to a disturbance of the meso-telencephalic dopamine which, *inter alia*, results in a relative predominance of cholinergic brain mechanisms<sup>6</sup>.

The second and more specific difficulty of the dopamine hypothesis is that dopamine receptor blockade sets in immediately after neuroleptic administration, whereas the onset of the antipsychotic effect in schizophrenic subjects has an 'irreducible' delay of several weeks<sup>2</sup>; during this time the dopaminergic system becomes supersensitive to dopamine (probably through an increase in the number of postsynaptic dopamine receptors)<sup>3</sup>. The therapeutic lag indicates that either the blockade of dopamine receptors produces therapeutically important adaptive changes in other, non-dopaminergic systems; or, independently of dopamine receptor blockade, chronic neuroleptic treatment — in contrast to its acute effect — influences directly other neuronal systems which may be crucially involved in the therapeutic response.

Third, the significance of the increase in number of dopamine receptors — a potentially very important finding — has been challenged. It has been claimed that the increase may be due to neuroleptic treatment (which most schizophrenic cases receive at some time during the course of their illness)<sup>7</sup>; in neuroleptic-free schizophrenics, a decrease in the number of the receptor sites has been reported<sup>8</sup>.

Fourth, in reference to the increase in number of dopamine receptors in the striatum/accumbens complex, observations in Parkinson's disease<sup>6</sup> as well as animal studies<sup>9</sup> point to predominantly motor (including stereotypy-related) functions of these brain regions. Thus, these increases in receptors may be related to the motor-stereotyped behavioural abnormalities often seen in schizophrenics rather than to the fundamental symptoms of the illness. Furthermore, symptoms of brain dopamine supersensitivity in the form of involuntary movements, which develop in non-schizophrenic individuals chronically receiving neuroleptics, have never been seen to be accompanied by psychotic symptoms<sup>10,11</sup>. This is significant in view of animal data showing that neuroleptic-induced brain dopamine supersensitivity is indeed accompanied by an increased number of D-2 dopamine receptors in the striatum/accumbens complex<sup>3</sup>. One may then justly ask: what is the precise functional significance of the extra dopamine receptors found in the basal ganglia of schizophrenic patients? Given so many problems unanswered by

the dopamine hypothesis, it seems that we do indeed need to take a close look at other possible neurotransmitter disturbances in schizophrenia.

## Noradrenaline — a neurohumor for all purposes

Noradrenaline is very widely distributed in the brain of mammals<sup>12</sup>. Comparatively few cells bodies located in the lower brainstem (in the rat, the locus coeruleus, the largest noradrenaline-containing nucleus, contains not more than about 1,500 neurones) give rise to fibre systems with unusually high degrees of collateral arborization, innervating the whole of the neuraxis. In the human brain, noradrenaline is a 'limbic monoamine' *par excellence*, more so than dopamine or serotonin<sup>13</sup>. The regions in which noradrenaline, on a relative basis, is the predominant catecholamine include the nucleus accumbens, nucleus of the stria terminalis, ventral septum, central amygdaloid nucleus, the paramedian thalamic nuclei and the mammillary body. Even in absolute terms, the noradrenaline innervation of the majority of subcortical limbic regions is, in the human brain, denser than their dopamine innervation; in human limbic cortex areas, the noradrenaline levels are, as a rule, equal to or higher than the dopamine levels<sup>13</sup>.

Noradrenaline is thought to play a part in several brain functions, including stress, rage, aggressiveness, locomotor behaviour, arousal, reward, memory, sleep-wakefulness cycles, neuroendocrine regulations as well as autonomic control (for example, temperature regulation and cardiovascular control<sup>12,14-17</sup>). Relevant to this diversity are physiological studies demonstrating that stimulation of noradrenergic fibres or iontophoretic application of noradrenaline will not only potentiate the effects of inhibitory (non-adrenergic) inputs to the neurones under study but also the effects of excitatory inputs<sup>18,19</sup>. Rather than being a classical neurotransmitter, brain noradrenaline may thus serve as a 'bias' adjusting, or 'gain setting' system, improving the quality of neurotransmission of both inhibitory and excitatory inputs to a given neuronal system<sup>12</sup>. This property enables brain noradrenaline to exert diverse, opposite effects on the neuronal systems with which it normally interacts. It is in this context, together with the widespread innervation pattern, that the wide diversity of functions proposed for brain noradrenaline has to be viewed. These characteristic physiological and anatomical properties of brain noradrenaline render it a suitable agent to influence, in a pervading and subtle manner, the activity of those brain centres whose disturbed functioning may play a part in the production of psychotic behaviour.

## Noradrenaline and schizophrenia

Are there any clinical observations implicating brain noradrenaline in schizophrenia? It is known that, in

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addition to their effect on dopamine receptors, neuroleptics block noradrenaline receptors<sup>20</sup> and specifically inhibit a noradrenaline-sensitive cyclic AMP-generating system in the (rat) limbic forebrain<sup>21</sup>. In contrast to the dopamine receptor blockade, the noradrenaline blocking potencies of neuroleptics, as measured in acute experiments or *in vitro*<sup>20,22</sup>, are not well correlated with their antipsychotic potencies (although recently a better correlation has been observed in a limited neurophysiological study<sup>23</sup>). However, since the antipsychotic potencies are assessed on chronic neuroleptic administration, it is not clear how relevant data on receptor blockade under acute conditions, or *in vitro*, may be.

A role for brain noradrenaline in schizophrenia may also be deduced from the studies of Kornetsky and his colleagues<sup>24-26</sup>. They showed that a significant number of schizophrenic patients are in a state of chronic hyperarousal and suggest that this is the result of a chronic overactivity of the neuronal systems in the brainstem reticular formation responsible for arousal and attention. They propose that neuroleptics are beneficial because they suppress this hyperarousal to levels consistent with optimum performance. In this context, it is important that neuroleptics effectively block the behavioural as well as the electroencephalographic arousal reaction produced by stimulation of the brainstem

reticular activating system<sup>27</sup>; and that noradrenaline is the monoamine most frequently implicated in the functioning of the brainstem reticular mechanisms responsible for arousal<sup>17,28,29</sup>. In view of the facilitatory effects of brain noradrenaline on neuronal information processing<sup>30</sup> as part of the amine's 'bias' adjusting properties, noradrenergic hyperactivity in schizophrenia would be bound to exert a critical influence on many brain functions. Is there any direct evidence for this possibility?

Recently, Farley *et al.*<sup>31</sup> measured the monoamine levels in schizophrenic brains and compared them with carefully matched controls. They found highly above-normal noradrenaline concentrations specifically in several regions of the limbic forebrain, including (in order of statistical significance) ventral septum, nucleus of the stria terminalis, nucleus accumbens and mammillary body. Significantly, all four examined cases were chronic paranoid schizophrenics. Confirmatory results have subsequently been obtained by other research groups. Thus, Kleinman *et al.*<sup>32</sup> analysed autopsy brain material from 26 cases with psychotic disorders. In the chronic paranoid schizophrenic group (10 cases), significantly above-normal noradrenaline (and 3-methoxy-4-hydroxyphenyl glycol [MHPG]) concentrations were found in the nucleus accumbens. Carlsson noticed increased noradrenaline levels in the lower

brainstem of paranoid schizophrenics<sup>33</sup>. Other research groups<sup>34-36</sup> found a significant elevation of noradrenaline levels in the cerebrospinal fluid of schizophrenic cases, with smaller or no changes in other psychotic categories<sup>34,35</sup>; paranoid schizophrenics showed the most significant differences<sup>34</sup>. All these observations indicate the existence of a significantly increased noradrenergic system function in the schizophrenic brain.

At present only speculations can be offered about the possible causes of the increased noradrenaline levels in the schizophrenic brain. In view of the unchanged noradrenaline/MHPG ratio in the schizophrenic accumbens<sup>32</sup>, denser than normal innervation of the affected brain regions seems a plausible possibility. A tempting suggestion is that noradrenaline terminals fail to regress during postnatal development<sup>31</sup>, given that a marked reduction in density of catecholaminergic innervation of the neocortex is part of a normal process during the early postnatal period in the rat<sup>37</sup>. The possibility seems particularly attractive as schizophrenia is usually an illness of adolescence. Denser than normal noradrenaline innervation of certain (limbic) brain areas could also stem from the heterotypic sprouting of noradrenaline terminals that has been seen in response to damage of other neuronal systems<sup>38,39</sup>. Recent computed tomography studies indeed suggest the possibility of morphological changes in schizophrenic brain<sup>40-42</sup>.

#### Noradrenaline may regulate the sensitivity of the dopamine system

In looking for a unifying explanation for the noradrenaline and dopamine abnormalities in the schizophrenic brain, it should be remembered that neither the noradrenaline system nor the dopamine system functions in isolation. Pharmacological evidence for noradrenaline/dopamine interactions has been known for some time<sup>43</sup> and a dynamic interrelationship between these two systems in the brain has been proposed<sup>44</sup>. Recently Tassin and his colleagues<sup>45</sup> have shown in the rat that damage to the noradrenaline fibre system innervating the prefrontal cortex prevents the development of denervation supersensitivity to dopamine of the D-1 dopamine receptor system (that is, the dopamine-stimulated specific adenylate cyclase) in the affected area. This, for the first time, provides a physiological mechanism for the noradrenaline/dopamine interrelationship in the brain, and demonstrates the controlling influence of the noradrenaline system on the sensitivity of at least one type of brain dopamine receptor. Thus, the idea appears not too far-fetched that there may exist a connection between the increased noradrenaline levels in the schizophrenic brain and the abnormality in the brain dopamine system.

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### The future for noradrenaline research in schizophrenia

So far, only anecdotal data seem available on the therapeutic implications of the research on brain noradrenaline in schizophrenia. It is known that a few 'atypical' neuroleptics (for example, thioridazine, clozapine) with low Parkinsonism-inducing (dopamine blocking) potency are strong noradrenaline receptor-blocking agents<sup>20</sup>. On the other hand, classical  $\alpha$ -adrenoceptor blockers, for example, phenoxybenzamine, do not appear to have any known antipsychotic activity (although no proper clinical trials seem to have been conducted). Beneficial effects in schizophrenic patients have been claimed for the  $\beta$ -adrenoceptor-blocking propranolol<sup>46</sup>, especially when combined with neuroleptics; this combined effect, however, may be due to pharmacokinetic interactions<sup>47</sup>. Recently, a definite antipsychotic effect of clonidine in schizophrenic patients free of neuroleptic medication has been reported in a controlled double-blind study<sup>48</sup>. Conversely, exacerbation of psychotic symptoms has been seen on withdrawal from chronic clonidine regimen<sup>49</sup>. One of clonidine's actions is to decrease the synaptic release of noradrenaline by stimulating the inhibitory  $\alpha$ -2

autoreceptors located on noradrenergic terminals<sup>50</sup>. Obviously, these potentially quite important observations stress the need for novel, more specific centrally acting adrenoceptor-active compounds. Interestingly, yohimbine is a fairly selective blocker of the noradrenergic  $\alpha$ -2 autoreceptors<sup>50</sup>, thus facilitating synaptic noradrenaline release; one may wonder whether the property of yohimbine to activate latent psychotic processes in some schizophrenic patients<sup>51</sup> is due to this noradrenergic effect. Imipramine also worsens schizophrenic symptoms<sup>52</sup> and is known to increase the synaptic availability of noradrenaline by blocking its removal (by re-uptake). Despite these interesting observations, it has to be admitted that the dopamine hypothesis still is generally held to be very attractive, mainly because of the good correlation between the dopamine-blocking and the antipsychotic potencies of neuroleptic drugs; however, as pointed out, this evidence is open to criticism. Obviously, much more extensive work needs to be done. The possible involvement of brain noradrenaline in schizophrenia opens new aspects of the neurobiology of this illness and also holds some promise that new pharmacotherapeutic approaches to this disorder (or group of disorders) will be found in the foreseeable future. □

activities. Determination of the three-dimensional structure of subtilisin by X-ray crystallography, together with knowledge of the sequence, led to the first example of this possibility. The active site of the subtilisin group of microbial serine proteases bears close similarities to that of the mammalian type although the conformations of the two families of enzymes have no features in common. The location of similar catalytic centres on quite different molecular frameworks suggests that the same biochemical problem has been solved in the same way by evolutionary pathways starting from unrelated ancestral proteins and genes. The story is made more fascinating by the fact that certain microorganisms are known to synthesize both the subtilisin and the mammalian types of protease.

In this issue of *Nature* (p.564), Nakamura and his colleagues provide another example of this phenomenon, commonly attributed to convergent evolution. They have determined the three-dimensional structure of a microbial ribonuclease which, like the pancreatic ribonucleases, has the ability to hydrolyse 3',5'-phosphodiester linkages, and compared it with the structure of bovine pancreatic ribonuclease A. The only feature in common is a three-strand anti-parallel  $\beta$ -sheet and alignment of this feature demonstrates the absence of any other conformational similarity and hence evolutionary relationship. However, not only is the framework different but the types of residue assigned to the catalytic site and their mounting on the framework also bear little relationship to the well known active site of ribonuclease A.

The place of one histidine is apparently taken by an arginine residue, while the contribution to the cationic nature of the site in RNase A from Lys 41 is absent in the microbial enzyme, the other neighbouring residue being a glutamate (assuming Lys 65 does not turn back towards the active site). The two histidines of RNase A are located on the S-peptide and the S-protein and are brought together by the association of these two structures which fold independently. In the microbial enzyme, the three functional residues are located one on each of the three  $\beta$ -strands making up the central hydrophobic core, which, on initial inspection, appears to possess the characteristics for a single nucleating folding unit. It will be interesting to compare the basis for nucleotide base specificity when enzyme-substrate complexes become available.

The elucidation of this structure by Nakamura and colleagues raises, therefore, particularly interesting issues of both enzyme structure and the evolution of catalytic activity. If the two nuclease activities arose by divergent evolution it would seem that the common ancestral protein was conformationally relatively simple and unstable and that modification could, and did, occur in more than one

## The evolution of enzyme activity

from Roger H. Pain

It is fairly safe to speculate that Darwin did not foresee the development and application of X-ray crystallography to proteins, so the idea of physicists contributing to the evolutionary debate would have caused him some surprise. Darwin was concerned about form and function at the macroscopic level. An interesting aspect of current progress in the field of molecular evolution is that the biophysicist is increasingly interested in shape and function at the molecular level as features which have been selected and conserved for various reasons.

Molecular shape, or three-dimensional conformation, is coded for by the amino acid sequence of the polypeptide chain which determines both the folded structure and the pathway by which the chain folds. The code appears to be highly degenerate, for clear similarity in chain folding may be seen between different proteins even when there is little conservation of amino acid residues. In several families of proteins, it is conformation which is primarily conserved, with the amino acid sequence being variable within the limits of its ability to code for the particular folding pattern.

There is a variety of degrees of correlation between protein conformation and the associated biochemical function.

Thus the mammalian serine proteases possess the same catalytic site but different binding sites on closely similar backbone conformations. Immunoglobulins of different classes and from different species comprise domains with highly conserved framework structures which are capable of supporting a wide variety of binding site residues. And a particular folding pattern, first recognized in the nucleotide-binding domain in dehydrogenases and frequently termed the Rossman fold, is now thought to occur in other proteins which do not bind nucleotides at all. The specific type of activity, therefore, is presumably not always responsible for the selection of a particular conformation. Whatever the properties of a folding pattern which lead to its selection — and these are by no means clear — a close link between conformation and activity is not mandatory.

A reasonable converse to the foregoing patterns of protein structure-function relationship would be that different conformations, necessarily coded for by quite different amino acid sequences, could support similar biochemical

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direction while preserving the enzymatic activity. Such modifications could have been neutral with respect to selection of the enzyme conformation, if selection operated solely on the catalytic function. The catalytic activity of an active centre is, however, unlikely to be completely independent of the nature of the framework. Another possibility is that the framework parts of the enzymes might have responded to the different environments of microbial and pancreatic ribonucleases, for example, in terms of stability and specific non-catalytic interactions. This would lead to selection based on conformation as well as catalytic activity. Such features may or may not

necessarily be of selective advantage at the present point in evolution.

The alternative process of convergent evolution can be envisaged as arising from two quite different polypeptides which both, by chance, possessed primitive nuclease activity which was selected for, with again the possibility of other factors being involved in the selection of the two different classes of ribonuclease. It is clearly difficult at present to choose between these alternatives. It would be an interesting challenge to the enzymologist, however, to investigate the existence of factors other than the operation of the active site in the functioning of groups of enzymes such as these. □

## Image processing in the analysis of art

from Gary W. Carriveau

DIGITAL image processing, which for many years has been used to bring out remarkable details in pictures taken by space probes, has recently been applied to the study of fine art. As reported at a recent meeting of art conservationists\*, these applications include the separation of superimposed images in oil paintings, the authentication of art works and the study

of the artist's technical procedures.

The first step in processing the image is to break it up into picture elements or 'pixels'. Each pixel is assigned a level of brightness or grey scale, normally in the range 0–255. (The human eye can only differentiate about 40 levels of grey; the computer's expanded range allows for more accurate computations.) A typical image has 1,024 horizontal pixel lines and 1,024 vertical pixel lines; the image is then composed of over one million individual

numbers.

Over the years many computer programs and algorithms have been developed and most image-processing laboratories can process images in a number of ways. Some of the most commonly used are: (1) enhancement of edges, accomplished by subtracting a fractional value of the laplacian transformation of the image from the image itself. This enhances and improves the contrast and the effect may be controlled by varying the fractional coefficient. This may be compared with over- or under-exposing a black and white photograph; however, the digital processing may be applied to specific regions of the image, it may be done as a reiterative process and it is fast. (2) Correction of grey-level differences in an image through grey-level histogram matching. This is especially useful in dealing with composite photographs made up of individual photo segments produced with varying lighting conditions. (3) Removal of certain distracting features in an image by using a nonlinear point operation or by subtracting that portion of the frequency spectrum of the Fourier transformation contributing to the distraction. (4) Simplification of complicated pictures, making them more interpretable by simply subtracting one or more parts from the composite image.

The pioneer efforts to apply these techniques to the analysis of art grew out of the Jet Propulsion Laboratory's 17 years of image-processing experience in the space

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'The Crucifixion' (left), a seventeenth-century oil painting by an unknown Flemish artist, was painted over another, completely different work (right) which depicts a man and woman in seventeenth-century clothing seated at a table. This was revealed by X-radiographic photography and computer processing by the Jet Propulsion Laboratory and the Los Angeles County Museum of Art. (Photographs provided by the Jet Propulsion Laboratory.)



programme, beginning with the early Ranger photographs of the Moon and continuing through the latest Voyager images of Saturn.

Image-processing methods were first applied in an investigation of bronze statues by Edgar Degas. The results were used to study the fineness of surface texture, and thus determine whether objects were recastings from an original sculpture. A second example dealt with a painting entitled 'Deposition', initially attributed to Van Dyck. A study of the inner structure of this painting using X-ray radiographs showed two overlapping features: a lower image of a sitting room (invisible to the naked eye) and the upper visible image of the deposition of Christ (see the figure). Image processing was then used to remove a distracting, complex pattern in the X ray, a consequence of the

grain of the wooden panel on which the painting was executed, and the two painted images were totally separated with enhancement and subtraction techniques. The Jet Propulsion Laboratory and Los Angeles County Museum of Art are also collaborating on image processing-assisted investigations of paper deterioration and conservation, especially concentrating on the care and handling of the Codex Hammer by Leonardo da Vinci.

Another project, linking the Detroit Institute of Arts, the Metropolitan Museum of Art, New York, and the Computer Science Department of Wayne State University, Detroit, uses processing to help interpret IR reflectograms from paintings, and in the Landesmuseum, Bonn, image processing is being used to decipher aerial photographs and so identify ancient sites for excavation. □

## Generation and propagation of acoustic gravity waves

from T.B. Jones

ONE significant way in which energy can be transferred from the lower to the upper atmosphere is by wave propagation. In particular, acoustic gravity waves (which have periods typically of 5–100 min) have long been observed by pressure sensors and by the effects of the waves on radio signals as they propagate through the ionized upper atmosphere. By extrapolating back from the observed travel paths, it has been found that thunderstorms are an important source of the disturbances. Recent radar observations<sup>1</sup> have now provided an explicit demonstration of this process.

A wide range of freely propagating waves can exist in the Earth's atmosphere. When compressibility forces determine the wave characteristics they are termed 'acoustic waves', with a lower frequency limit at the 'acoustic cut-off frequency'. At lower frequencies, buoyancy forces play a major part in the propagation of the waves, hence in this frequency range the waves are referred to as 'internal gravity waves', with an upper frequency limit at the 'Brunt-Vaisala frequency'. Such acoustic gravity waves (AGWs) are common in most regions of the atmosphere where they manifest themselves as disturbances, having a wide spectrum of frequencies and scale sizes, imposed on the ambient wind, density and temperature structure. The linear propagation characteristics of these waves have been described by many authors (see ref 2., for example) and are now well understood.

AGWs in the lower troposphere near the

atmospheric boundary layer have been observed for many years using experimental techniques such as microbarographs, wind sensors and acoustic sounding. At higher levels in the troposphere and stratosphere they have been detected by sensors carried on balloons, aircraft and rockets.

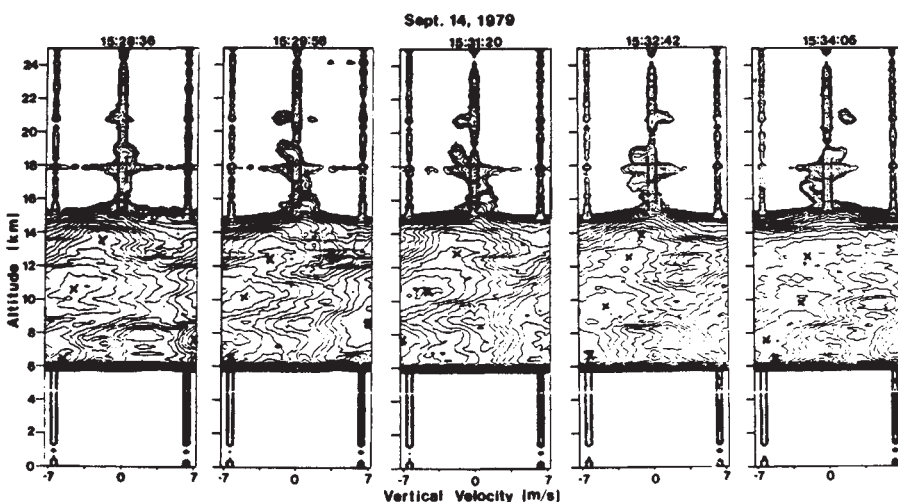
At heights above 60 km, the atmosphere becomes ionized and the free electrons act as tracers for the atmospheric waves since their displacement can readily be measured by various radio sounding methods. These include HF Doppler sounding<sup>4,5</sup>, ionosondes<sup>6</sup> and UHF backscatter<sup>7-10</sup>.

More recently VHF and UHF radar<sup>3</sup> and lidar techniques have been developed

which allow accurate remote sensing of atmospheric waves through the troposphere, stratosphere and mesosphere. The existence of several types of AGW sources together with the ability of these waves to transport energy and momentum both horizontally and vertically suggest that they provide an important coupling mechanism between various levels of the atmosphere. Energy is absorbed from the waves by linear processes such as viscous dissipation and newtonian cooling. In addition, more complicated processes exist, such as the interaction with the ambient winds to produce critical coupling and nonlinear dissipation mechanisms. The importance of these nonlinear effects increases with height because in order to maintain wave energy density against the decreasing gas density, the amplitude of the vertically propagating AGW increases exponentially with altitude.

At high latitudes the major sources of AGWs are associated with auroral phenomena such as changes in the position and intensity of the electrojet and particle precipitation events. Large-scale waves are mostly of auroral origin and their occurrence is closely correlated with indices of geomagnetic activity such as  $Kp^{11,12}$ . At mid and low latitudes the major sources of AGWs are thought to be in the lower atmosphere and there is considerable evidence for tropospheric sources associated with severe weather conditions. The source locations can be determined from observation of the AGW characteristics by means of a reverse-ray tracing analysis in conjunction with a model atmosphere. This analysis has been applied to ground-based microbarograph and acoustic sounding observations<sup>13-15</sup> and to the ionospheric response<sup>16,17</sup> and the results confirm that severe thunderstorms can generate AGWs.

The likely generation mechanism, first suggested by Pierce and Coroniti<sup>18</sup>, is penetrative cumulus convection and this hypothesis is directly supported by Larsen



Contour plots of reflected power as a function of Doppler shift (vertical velocity) and height above mean sea level. See the text for further explanation. (From Larsen *et al. Geophys. Res. Lett.* 9, 571; 1982.)

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*et al.*'s<sup>1</sup> UHF (430 MHz) radar measurements at Arecibo, Puerto Rico. The radar observed vertically propagating AGWs being launched into the lower stratosphere when the tops of cumulus clouds penetrated the tropopause.

The figure shows five consecutive profiles with each frame representing a contour plot of power versus Doppler shift (vertical velocity) and height above mean sea level. The lower height limit of the measurements was 5.7 km and the vertical bars at  $\pm 6 \text{ m s}^{-1}$  are due to interference from the 60 cycle mains supply.

The height of the tropopause on this day was 14.5 km and the broad spectra between 5.7 and 14.5 km are due to a cumulonimbus cloud within the sampling volume. Above 14.5 km there is an oscillation in the vertical velocity with a wavelength of 7 km. The period is approximately 6 min. Examination of the zero crossings associated with the wave as time progresses through the observing sequence indicates a downward phase progression. For a vertically propagating gravity wave this implies an upward propagation of energy and is consistent with the suggestion that a gravity wave is generated once the cumulus cloud reaches the boundary of the more stable atmosphere.

The velocity time series at several heights have been examined and subjected to Fourier analysis. The wave frequency is found to change with height. It is very close to the Brunt Vaisala frequency below 16 km, which is close to the source of the wave if the wave is generated at the bottom of the tropopause boundary. Between 16 and 18 km the wave frequency and the Brunt Vaisala frequency are both increasing. The wave frequency becomes constant above 18 km. Thus, the wave frequency tends to follow the Brunt

Vaisala frequency profile for thunderstorm-generated gravity waves.

For heights above 18 km the wave has been examined in terms of its depression characteristics. Two solutions of the dispersion relationship were found corresponding to horizontal wavelengths of 2.17 and 14.3 km respectively. The longer wavelength was found to be the more likely solution since the attenuation associated with the critical level should make the shorter wavelength impossible to detect above 16.3 km. Theoretical studies of penetrative convection undertaken by Townsend<sup>19</sup> and by Stull<sup>20</sup> support this mechanism as a source of AGWs.

The effects of more severe tropospheric disturbances, such as exist during hurricane conditions, have been investigated theoretically<sup>21,22</sup> and experimental evidence of AGWs produced by hurricane Eloise reported<sup>23</sup>. Since the AGW propagation velocity in the upper atmosphere is greater than the speed of the hurricane, it is possible to develop a

warning system for such storms based on their ionospheric signatures<sup>24</sup>.

Another meteorological source of AGWs is associated with the wind shear instability in the tropospheric jet stream<sup>25</sup>. Sources close to the jet stream have been located by reverse-ray tracing from UHF radar observations of AGWs<sup>26-28</sup>. Theory indicates that, although the wind shear is unstable to horizontally propagating waves, their growth rate is much less than that of the non-propagating Kelvin-Helmholtz instability. Observations of VHF backscatter<sup>29</sup> confirm the production of the Kelvin-Helmholtz instability during conditions of strong wind shear.

Attention has also been given to topographical features such as steeply rising mountain ranges, since it has long been known that they generate wave-like structures in the air flowing over them<sup>30</sup>. These 'mountain lee waves' are, however, stationary with respect to the ground when the air flow is steady<sup>31</sup> and are unlikely to produce freely propagating waves. □

## A novel class of wasp viruses and insect immunity

from Peter Faulkner

A NEW class of viruses, discovered in the calyx fluid of parasitoid wasps, appear particularly intriguing on at least two counts. First, the particles contain genomic DNA in a novel packaging arrangement and second, as revealed in a recent report<sup>1</sup>, the viruses seem to have a role in abrogating the immune response of the target insect and permitting eggs of the parasitoid to develop normally.

Indigenous viruses exist in the ovaries of numerous species of parasitoid wasps which belong to the families Braconidae and Ichneumonidae<sup>2</sup>. They replicate in the nuclei of calyx cells, an epithelial tissue located between the ovarioles and oviducts of the parasitoid. Virus particles bud from calyx cells into the lumen of the oviduct and as a component of the calyx fluid they are injected, together with an egg, into the target caterpillars. Successful development of parasitoid eggs depends on the presence of the virus which acts to suppress the immune response of the target insects and thus prevent encapsulation and inactivation of the egg. Edson *et al.* call the phenomenon 'obligatory mutualism' between a virus and a eukaryotic organism.

In a series of reconstruction experiments, virus particles were first separated from the rest of the calyx fluid and then the components were injected with eggs into target tobacco budworm caterpillars. When eggs of the parasitoid wasp *Campoletis sonorensis* were injected with saline into the caterpillars, all of the

eggs were encapsulated by the target insect, which was thus protected from the parasitoid. Injection of a virus separated from other calyx fluid components by gradient centrifugation effectively suppressed the encapsulation. The presence of the living virus was apparently necessary as when it was inactivated by UV radiation, encapsulation occurred.

Surveys by Krell, Stoltz and Vinson<sup>3,4</sup> over the past few years have confirmed the ubiquitous presence of virus particles in calyx fluids of parasitoid wasps and although the fine details of virus morphogenesis have not yet been observed, it is known that when the particles are injected into a new host, they fuse with cell membranes, become uncoated and enter the nucleus<sup>5</sup>. Enveloped particles of varying morphology have been isolated from braconid wasps but those from the ichneumons appear to be rod-like enveloped nucleocapsids and, at least in *C. sonorensis*, are regular in size. The parasitoid viruses probably fall into a new taxonomic class of animal viruses since their genomes are multipartite and consist of a set of covalently closed circles which, in the case of *C. sonorensis*<sup>6</sup>, has a molecular weight of  $4-13.6 \times 10^6$ , and in the braconid *Apanteles melanoscelus*<sup>4</sup>  $2-25 \times 10^6$ .

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Some elegant studies by Krell and co-workers have recently further characterized the DNA of *C. sonorensis*<sup>6</sup>. Since the viruses cannot be grown in tissue culture, it was necessary to dissect ovaries from 50 to 100 female wasps as a preliminary to each virus purification step. DNA from purified virus was then analysed on CsCl<sub>2</sub> ethidium bromide density gradients and separated into superhelical and closed circular fractions, both of which were analysed by agarose gel electrophoresis. Southern blot hybridization of superhelical bands excised from gels and subsequently cleaved with restriction endonucleases led the investigators to conclude that most of the bands were made up of unique DNA sequences. However, the different size classes of the covalently closed viral DNAs do not appear to exist in equimolar

concentrations, which may indicate that sub-classes of the virus co-exist in nature.

The work of Krell and his associates represents the most complete molecular study of the ichneumonid viruses so far and their results clearly set the stage for further investigations of the nature of the replication cycle of this class of multipartite DNA viruses as well as offering yet another model to invertebrate immunologists to study the complex immune system in invertebrates. □

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## Gaps in the fossil record

### Fossils and stratigraphy

from Robert M. Schoch

IN a recent *News and Views* article<sup>1</sup>, Schindel expresses the view that, given extremely careful stratigraphical sampling of fossil sequences, "the patterns distilled from these relatively short, complete sequences can be read directly, and can be calibrated in years using short-term sedimentation rates". In this way, Schindel apparently believes that one may directly study evolutionary rates in organisms. But this methodology contains a fundamental assumption — it relies on the stratophenetic method of phylogeny reconstruction<sup>2</sup> that many regard as unsound<sup>3</sup>. Schindel<sup>1,4</sup> assumes that samples of fossils found in stratigraphical sequence form monophyletic, evolving lineages of ancestors and descendants without providing documentation in the form of 'sound phylogenetic analysis' of the taxa involved<sup>5</sup>. However, in some sequences, local extinctions and ecological re-entries can be documented<sup>6</sup> so that separate populations, whose genetic relationships to one another are not necessarily known, may be sampled in successive layers. Indeed, it has been cogently argued that ancestor-descendant relationships cannot be objectively recognized in the fossil record<sup>7</sup>. If this is

true, it is impossible for palaeontology to address the problem of the nature and process of microevolutionary change. □

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### Deme histories are not species' histories

from David E. Schindel

SAMPLING any fossiliferous outcrop on a centimetre-by-centimetre scale has the potential to provide detailed complete population histories over geologically short time spans (see, for example, refs 8,9). Such patterns of morphological history are, however, representative only of local populations, not of a species as a whole. The history of a species is the aggregate of all the histories of local populations, which can be very different and yet still remain within the limits of intraspecific variation. The variation can stem from slight differences in habitat conditions, the vagaries of gene flow at the local level, random dispersal and isolation events (operating on a regional scale), and the eventual non-synchronous demise of local populations caused by habitat change or destruction (operating locally and regionally on a geological time scale)<sup>10</sup>. These sources contribute to seemingly random morphological walks that still lie within the realm of evolutionary stasis. Clearly, sorting out these different sources of variation requires a sampling scheme with replicate sampling of outcrops found elsewhere in the local area, and in other regions. Only when the limits of local and

inter-regional variation are known can ancestor-descendant relationships be reconstructed. This point is applicable to cladistic analysis as well as to other less formal research programmes. Thus, the morphological history of a species is more complicated and less easily read than the morphological history of any local population. Microstratigraphical sampling speaks only to local histories, not the global history of a species.

Can microstratigraphical sampling provide direct evidence for ancestor-descendant relationships? I think not, for the most part. The discontinuous nature of sediment accumulation gives rise to a catch-22 situation. Short bursts of local sediment input create stratigraphical sequences that are short in scope, and high in acuity and completeness. But, as Ager<sup>11</sup> has noted, "the finger of sedimentation moves" through time, so local sections are very incomplete with respect to geological time. Combining the morphological patterns distilled from different local sections into one composite section (for example, ref.12) destroys all the details to be recovered from individual short, complete intervals. Estimates of completeness for the best studied cases<sup>1</sup> indicate the likelihood that any moment in time is unrepresented in most places. But each local hiatus might include time sufficient for the deposition of twenty separate sedimentary wedges. If local sedimentary wedges somehow could be ordered in the correct temporal sequence, it would be possible to reconstruct a species' history through a geological time interval more completely, but such a task is beyond the present limits of pre-Holocene dating techniques. For the present, it seems best to present local patterns side-by-side, rather than in a single composite column constructed by forcing local sections together using only stratigraphical position within a formation as a temporal correlation tool.

Here's the catch: in order to know ancestor-descendant relationships directly, one would need to know most, if not all, of the local population histories at the microstratigraphical level, and have them arranged in the correct order. Yet it is impossible, for the present, to reorder all the local populations (in fossil form) into the correct temporal mosaic. Until magnetostratigraphy, biostratigraphy and isotopic dating techniques are refined below the scale of hundreds of thousands of years, stratigraphical information will be of secondary value in reconstructing phylogenies. Minimum ages for first appearances can be established, approximate branching points can be estimated in time and space. Microstratigraphical sampling will provide only local population histories, each of which will be very unlikely to record a speciation event within a small, isolated population. □

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# NEW JOURNALS REVIEW

## Making sense of journal publishing

*With cut-backs in library budgets it is hard to understand why new journals continue to appear. Here Robert Campbell discusses the economics of journal publishing and the prognosis for this part of the scientific literature.*

THROUGHOUT the world library budgets are being cut; although books were the first to suffer, this inevitably also means cancellations of subscriptions to periodicals. Yet despite a difficult market publishers are launching new journals. To many this appears to make no sense — there already seem to be more titles than any researcher can cope with, let alone libraries purchase. With the prediction of even more severe cuts in library acquisitions next year, publishers appear to be pushing the research journal towards a crisis in which a huge number of titles could run into deficits thus forcing widespread and abrupt termination of publication. The present system for the communication of primary research could be seriously disrupted, and it should be borne in mind

that the activities of academic societies are often subsidized by profits from the societies' journals.

But the publishers' apparently irrational behaviour can be explained. For some years the market for research journals has been static with circulations after the first five or six years showing little growth. Indeed, some of the major journals have sustained small annual losses in circulation of around 2–3 per cent as organizations rationalize their holdings. Sometimes libraries have spent part of the funds saved in this way by taking on new titles more suited to their current interests. The total market, therefore, has been static but there has been sufficient movement within it to encourage publishers to launch new titles as the only way of gaining a larger share, given that it is difficult to raise the library circulation of existing titles. A journal with 500 library subscriptions, or even fewer, can be economically viable.

The other way of taking a larger share of the market is to expand a journal by publishing more pages and raising the price accordingly. This explains why the subscription rates of many journals have increased faster than the retail price index or other indicators of inflation such as the printers' index (Fig. 1). What is not widely appreciated is that in terms of price per page journals have performed well in relation to inflationary increases in costs, as is shown in Fig. 1; but if circulations start to drop significantly sharp rises in subscription rates will be necessary, putting further pressure on libraries unless other sources of income can be developed.

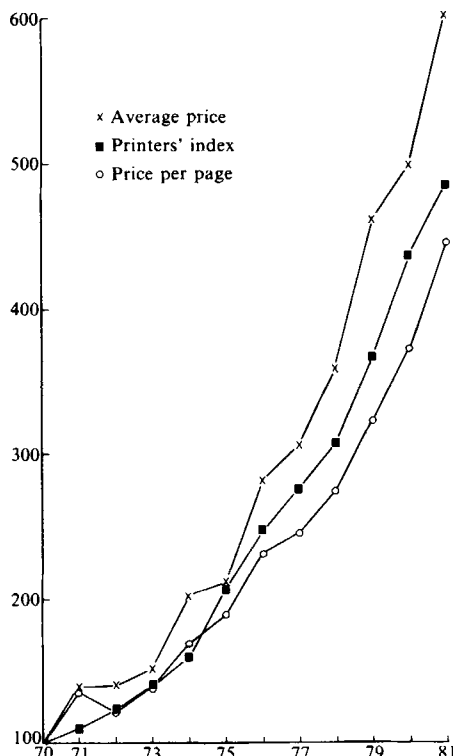
At present a few research journals are heavily subsidized by advertisers but the vast majority rely on subscriptions for 95 per cent of their income, the remaining 5 per cent coming from the sale of back issues and offprints. The secondary distribution of papers as photocopies by document delivery centres such as the British Library Lending Division and through interlibrary lending systems brings little or no income to the original copyright holder. As long as subscription incomes can support publication this has been annoying to publishers but not disastrous; in the next two or three years the situation could be entirely different with the survival of many specialized journals depending on gaining

income from all uses made of their copyright material.

We have, therefore, the possibility of a confrontation between journal publishers and the library community. Both sides have a case. As implied above, publishers must now demand some income from the secondary distribution of their material to maintain publication. On the other hand, libraries faced with successive cuts feel that they cannot afford to pay copyright fees saying that this would further reduce the service they give to users as they would have even less to spend on subscriptions. They have argued that in any case photocopying cannot be proved to reduce the circulation of a journal and thus harm it financially; there appears to be no firm evidence either way.

The United States Copyright Office of the Library of Congress is trying to find a fair solution to this problem. As part of an effort to assess whether Section 108 of the US Copyright Act of 1976 which relates to photocopying has achieved the intended statutory balance between the rights of creators and the needs of users of copyright works, the Copyright Office commissioned a major survey on libraries, publishers and photocopying. This was carried out by King Research and recently made public.

To no one's surprise the report reveals that 70 per cent of the US public, academic, federal and special libraries have heavily-used photocopying machines and more than half have photocopying machines within walking distance of the library and probably, therefore, outside the librarian's control. Apparently 6.5 million serial interlibrary lending requests in 1981 were filled with photocopies; an overall increase of 9 per cent over 1976, but with public libraries actually experiencing a decline and academic libraries showing a 16 per cent increase. Generally the situation has not changed radically between 1976 and 1980 but within the library system there appears to have been little growth in activity amongst public libraries and those serving commercial organizations, while academics appear to have been less affected by the economic climate. For example, academic libraries reported making 24.1 million photocopies in 1981, a 45 per cent increase over 1976. But, of course, in 1981 the real impact of the



**Fig. 1** Graphs showing the rise in the average subscription rate of a sample of life sciences journals (x) and the rise in the price per page of the same sample (o). The middle line (■) shows the rise in printers' prices based on an index supplied by the British Printing Industries Federation which has moved up in much the same way as the retail price index.

Reagan administration and the economic recession was only just being felt by the academic sector. Another survey in two years' time could reveal a very different picture.

In the part of the survey relating to publishing it was found that US publishers produced a total of 20,400 serial titles in 1980, an increase of approximately 21 per cent over the 16,900 serial titles published in 1976. The most dramatic change was in the number of article reprints sold, rising from 16.2 million in 1976 to 34.4 million in 1980 — a clear demonstration of the trend towards using copies of single articles rather than whole issues.

One curious result of the survey was the huge discrepancy between interlibrary loan requests received and the much smaller number placed, which suggests that librarians often try two or more other libraries to find a source. This may partly explain the high "real" cost of an interlibrary loan, estimated by King Research in an earlier study to be between \$15 and \$20 for an article. In Britain the system is more efficient in that librarians almost automatically go to one central source, the British Library Lending Division which through economies of scale is able to supply photocopies at low rates.

It is likely that researchers faced with declining library holdings and ever more titles will increasingly rely on copies of single articles identified through secondary services such as *Current Contents*, abstracts journals and now on-line searches. But they are supplied by a relatively cumbersome system where a source often has to be found at some expense, and the actual journal is taken off the shelves and photocopied with the document then going through the mail for delivery through the user's library.

Publishers and librarians are, therefore, looking towards new systems of document delivery based on information technology. For example, it is possible to store the full image of each page in compressed digital form on optical discs for retrieval and rapid print out. It is also possible but not yet economically feasible to transmit the digital form via a satellite for printing out in the user's library. Such is the progress of information technology that the late J.D. Bernal's dream — making available to individuals only copies of those articles in which they have declared an interest — may well become a reality within several years. A more efficient form of document delivery now made possible by the new technology should enable document delivery centres to supply libraries quickly and at lower rates, yet still at sufficient margins to enable them to pass back vital copyright revenue to the original publishers. The emergence of new titles catering for new needs and the survival of existing titles could depend on it. [□]

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## New journals June 1980 to May 1981

THE new journals review issue is intended as a service to potential subscribers (especially librarians) and authors, and also to publishers who may welcome comment on and publicity for their infant journals. How informative and comprehensive a survey these pages provide depends in part upon the reviewers, but also upon the cooperation of publishers. Unfortunately, this is not always forthcoming; not through obstructiveness but perhaps because of insufficient interest in many promotions departments.

Criteria for journals to be considered for inclusion in this issue were circulated to publishers earlier this year and were also printed in *Nature*. They were that:

- (i) the first number appeared, or the journal was re-titled, between June 1980 and May 1981 (although journals not covered in the previous review issue — *Nature* 293, 341–369; 1981 — were also considered);
- (ii) the journal appears at least three times a year;
- (iii) the main language used is English;
- (iv) where possible four issues should be made available for review, the first, the most recent, plus two others.

The 1981 journals review issue covered new publications appearing up to May of 1980, and the second cut-off date, May 1981, allows for enough issues of a journal to have been published for a reasonable sample to be available for judgement. Equally a spread of four *distinct* issues is taken as being the minimum on which reviewers' comments can be based.

Several journals known to satisfy the above criteria were not submitted for review, or arrived only in August or September, and therefore are not covered in this issue. And those publishers who sent in four identical issues of, for example, Vol. 1. No. 1, are less likely to find their journal reviewed here.

Disappointed editors should note, however, that it proved impossible to find reviewers for some doubtless worthy journals and that other titles were considered to be of marginal interest to *Nature's* audience. Of the larger publishers Elsevier Biomedical and Alan R. Liss are to be thanked for their speed and efficiency in supplying the material requested.

Although not comprehensive, over 60 journals are reviewed. The brief given to reviewers was to limit themselves to comment on the publications sent to them for review, and to avoid discussion of general questions of periodical publishing.

As last year, the preponderance of journals in the biological sciences reflects the bias of the material submitted for review and the shortest possible abbreviations of titles have been used. Opinions expressed in the reviews are based on a sample, and

apply to mid-1982 at the latest. Details of editors and frequency of publication, and the basic annual subscription rates appearing at the top of each review are given in most instances for 1983. These details are not complete in all cases, and readers *should check subscription rates in particular with the publisher concerned*. The reviews of journals begin on p. 497. [□]

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# What's news in biotechnology?

*The rise of biotechnology has brought with it a welter of publications all competing to provide rapid information. Julian Davies here surveys the field and comments upon this form of publishing. Further details of the publications are given on pp.495-496.*

WITHIN a few decades biotechnology has moved from an ancient industrial occupation for the production of beer, wine and bread, through to embrace the production of antibiotics, amino acids and so on, and now to one of the hottest areas of action in academic, industrial, financial and legal circles. The impact of this scientific bandwagon on university-industry relationships and the investment business has excited much comment and speculation, at times to the point of frenzy. Worldwide, there are now more than 200 companies involved in the application of genetic engineering to biotechnology and this explosion of commercial activity has been reflected by the publishing world. One can imagine the urgent editorial discussions: "We must get something on biotechnology".

So, coincident with the development of new biotechnology companies, has come the launching of a series of books and



journals, news-sheets and other more ephemeral literature. They provide on the one hand informed articles on the subject, written by scientists for scientists, and on the other abstracts, patent digests, news snippets and (undoubtedly) many rumours to enable the various companies to learn about successes and failures of the opposition — who is investing, who is the newest recruit from academia, what is the latest market analysis, how is government reacting — and keep their subscribers completely in the picture about "spectacular developments in genetic engineering". Biotechnology, like microchip and computer development, is an industrial research development race; it is essential to be aware of competitors and of what they are doing.

There are now some 30 publications that provide, in one way or another, rapid information on biotechnology, with more appearing almost weekly. As titles in the table (pp.495-496) show, it must be getting difficult to devise fresh names for these periodicals. Few of the titles are particularly original and most are a

permutation of "Bio", "News" and "Tech(nology)". Hence *Biotechnology News*, *Biotech News*, *BioEngineering*

## McGraw-Hill's BIOTECHNOLOGY NEWSWATCH

*News* and *Biotechnology Newswatch*! There is evidence of cloning here since many tend to report the same kinds of events in the same format.

They can be grouped into three types: the true magazines (*Biofutur*), the abstract and "intelligence" networks (*Telegen Reporter*) and what might be called the gossip sheets (*Biotechnology Newswatch*). They offer different formats, services and viewpoints, and probably fulfil different purposes. Very few of them would find their way into a university library. They are often extremely expensive (for example *Biotech-Update* offers ten issues a year for \$95, the first issue only ten pages long; *Biotechnology News* comes out fortnightly and costs \$210 in Europe for an eight-page issue) and most are designed to serve the needs of those who want to have some idea of what is going on without knowing details of the laboratory nuts-and-bolts side of the business. They cannot be considered journals, none carrying primary reports of research, but instead in the main present meeting highlights, press reports and hearsay in much the same way as a newspaper gossip column.



Reading comments about one's own company can be amusing; often they are misleading but they usually contain sufficient germs of truth to indicate that some facts are reported. In such a rat-race industry, members of business development groups must edge towards the post-room at the prospect of receiving the latest issue of one of the biotech gossip sheets. It must be difficult to avoid the feeling that you cannot afford *not* to subscribe — you might miss something.

The publishers of the biotech newsletters are not generally well-known and very few appear to have any reputation in science publication. This is probably indicative of the entrepreneurial and

risky nature of the exercise; most, like the small companies they report on, are based in the United States. We have not yet seen the end of this publishing wave since it should be an easy way to make money. Publishing costs are probably not high, since like most church and club newsletters one needs only a source of cheap paper, typesetting (optional — camera ready copy is the norm) and printing machines, and one or two people who know where to look for the gossip. Newsletters fall into the category of low-investment, low-volume, but high-cost commodities. Provided that

## Industrial Biotechnology

each biotechnology company is panting for news of its competitors, these publications can be launched at will, produced irregularly, but still sell.

It is thus all the more surprising to see McGraw-Hill (*Biotechnology Newswatch*), a staid, well-established, scholarly book publisher, entering into the fray with their scientific variant of the parish newsletter. Apparently, McGraw-Hill does have experience of this kind of venture in the literary field, but their effort in biotechnology is not especially impressive. Nonetheless, it has the advantage of a good name, firm financial backing and is incorporated into a Japanese version. All of this not only helps to establish the credibility of *Biotechnology Newswatch* but may also ensure its longevity in a cut-throat business.

A number of the biotechnology publications covered here are relatively subject-specialized. For example, *Biomass Digest*, *Biomass Bulletin*, *Agricultural Genetics Report*, *Biotechnology Law Report*, *Radioassay-Ligand Assay News* and *Frontiers in Immunoassay* appeal to different and much more restricted audiences than something like *Genetic*



*Engineering News*. Several are little more than abstracting services; however *Telegen Reporter* and Derwent's *Biotechnology Abstracts* both make a good job of this. *Telegen Reporter* is well established and somewhat more comprehensive in that it covers all sources of biogossip, including other biotechnology newsletters, meetings

## APPLIED GENETICS NEWS



and marketing reports. Notably, it also includes an excellent index. However, one has to pay for this excellence, at \$895 a year for 12 issues compared to its closest competitor, *Biotechnology Abstracts*, at £275 a year in twice-monthly issues. At the other end of the spectrum is *Biotechnology Information*, published by Teesside Polytechnic, presenting titles only, but gratis.



With this flood of information one must expect, and finds, enormous overlap and repetition. This is true in some cases for the same publication; for example, the second issue of Vol.1 of *Abstracts in BioCommerce* (a competitor of both *Telegen Reporter* and *Biotechnology Abstracts*) unnecessarily duplicates many of the



abstracts reported in the number 1 issue. This publication is well edited, cheap and comes from Celltech and IRL Press, an organization with a great deal of experience in scientific journals and abstracting. *Abstracts in BioCommerce* is new (like *Biotechnology Abstracts* it first appeared only this summer) so it does not yet have the impact of the other IRL abstract journals; nor, however, does it have the broad coverage and authority of *Telegen Reporter*. *Abstracts in BioCommerce* would do well to expand its brief to include more scientific information or, at least, to provide more background in its abstracts.

None of the publications has much to say about the ancient and conventional biotechnological industries — everything has to be new. It would have been refreshing (and useful) to read of the state of the wine harvest in Germany or to learn something about a new cheese culture developed in France. Priority in reporting doesn't seem to be important and it is unusual for the authors of articles to be identified (*Genetic Engineering News* and *Biotech Quarterly* are among those that do this). Few of the publications offer any special services; for an extra fee *Telegen Reporter* will provide

### Agricultural Genetics Report

edited by PETER W. DORFMAN

copies of any articles abstracted and *Biotechnology Abstracts* has an online computer database available. Equally, almost all of them are one-way news sources and are not concerned with opinion or communication, in the sense that there is no exchange between the readership and the publication. In this respect *Biofutur* and to a lesser extent *Industrial Biotechnology*, *Genetic Engineering News*, *Biotech News* and *Biotech Quarterly* offer their subscribers something a little bit different with letters from readers, book reviews and commentary.

It is clear that unless one is obsessed with missing something, there is no need to subscribe to more than two or three of these



World Pharmaceutical News

May 12th, 1982 No 682

publications. As is usually the case, you get what you pay for and the more expensive of them provide more extensive coverage, better indexing and extra services. All give useful information but to greatly varying extents.

Two deserve special mention. *Biofutur* is the leading glossy contender and provides good review articles and nice diagrams in a *Scientific American* format. It includes pithy and well-informed comment on commercial applications of genetic engineering but is not always up to date on biotech gossip. It is well written, but in French, and thus may have a very restricted readership. *Scrip*, the long-standing doyen of pharmaceutical news, has moved with the times and includes comments on most of the useful commercial moves of genetic engineering as applied to the pharmaceutical industry; *Scrip* is well indexed, which is not the case for many of these publications. In general it is very difficult to trace any information in back issues. For those professionally interested in biotechnology, both commercial and scientific, a

### Biotechnology Patent Digest

good index is essential if these publications are to be used as reliable sources of reference; the absence of an index tends to heighten the impression that they are supposed to be throw-away items that one scans for rumours and then discards when one learns that a competitor has or has not come up with another clone, or another product, or some investment.

How many of these periodicals will survive? If the biotechnology bubble is bursting, as some doomsayers would have it, will there be a lot of shredded paper? Very few of the publications carry any form of advertising to be the financial crutch during hard times — *Genetic Engineering News* is the leader in this respect. Will this aid its survival as the commercialization of genetic engineering becomes more commonplace and there are fewer hot items to report? In this context it is interesting to note that Mary Ann Liebert of New York publish no less than four separate newsletters in this area (*Biotechnology Press Digest*, *Genetic Engineering News*, *Agricultural Genetics Report* and *Biotechnology Law Report*).



MAY 1982 3

This is what is known as hedging one's bets and it seems likely that *Biotechnology Law Report* will have some contentious issues to discuss when the first patent disagreements begin to surface. This publisher has not only hedged its bets, but has put more eggs in the biotechnology basket than most. Mary Ann Liebert also puts out the *Journal of Interferon Research*, *DNA*, *Hybridoma* and *Lymphokine Research News* among others, and as an indication of willingness to face the music *Opera Arts* is listed among a broad spectrum of publications.

### BIOTECHNOLOGY PRESS DIGEST

May 1982

ISSN 0276-9128

VOLUME 1, NUMBER 1

Since the biotech newsletters and abstract services are here, presumably to stay for the time being, which of them can be recommended (assuming that one has to participate in the genetic engineering information rat race)? Given such permutations of title, content and format, and with the sure knowledge that more clones will be appearing, what should the serious reader do? It is in fact not hard to separate the sheep from the goats, since there seem to be so many goats.

At risk of inciting some discontent from a few of the good publishers involved, I will stick my neck out. For lively and timely presentations of both news and rumours, it is obvious that *Genetic Engineering News* has the lead; it may not be the first with the news because of its publication schedule (six times a year) but along with *Time* or *Newsweek* it makes good airplane or bedtime reading. *Scrip* is quick and easy to

### Practical Biotechnology

read, appears frequently and gives the most comprehensive information on all company activities. It is a bit thin on genetic engineering but it provides the right kind of background information for business and management groups. For serious literature searching and biotechnology news, then *Telegen Reporter* has no serious rival; however, Derwent's *Biotechnology Abstracts* is a reasonable replacement at lower cost if one only wants reports from the scientific literature. And last, *Biofutur*, with its French, intellectual demeanour, can be recommended for your coffee table.

Finally, it is worthwhile mentioning that scientists involved in genetic engineering should not need these publications. In spite of advertising claims to the contrary, anyone who reads the scientific literature proper, attends meetings and (above all) gossips with colleagues will catch the hot items long before the newsletters.

Julian Davies is Research Director of Biogen SA, Geneva.

# What's news in biotechnology? Publication details for 1982\*

| Publication                                                     | Publisher                                                                                               | Issues per annum | Subscription rate per annum                    | Comments                                                                                                                                                                                                                                              |
|-----------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|------------------|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Abstracts in BioCommerce                                        | IRL Press<br>PO Box 1<br>Eynsham<br>Oxford OX8 1JJ<br>UK                                                | 24 in 2 vols     | £60 (UK),<br>\$120 (USA)                       | Out of Celltech by IRL (who publish <i>Nucleic Acids Research</i> , <i>Microbiology Abstracts</i> etc.). Well presented and punchy. Abstracts English language newsletters, UK newspapers and some technical magazines; not patents or US newspapers. |
| Agricultural Genetics Report                                    | Mary Ann Liebert Inc.<br>500 East 85th Street<br>New York<br>NY 10028<br>USA                            | 6                | \$90 (USA), \$106 (elsewhere)                  | Mostly gossip, especially of and from meetings. Goes for headlines such as "Harvard's Bogorad to study corn DNA clone in algae".                                                                                                                      |
| Applied Genetics News                                           | Business Communications Co. Inc.<br>PO Box 2070C<br>Stamford<br>CT 06906<br>USA                         | 12               | \$250                                          | As above, but change title to "Interferon produced in yeast cells by collaborative research". Also has agricultural news, so some overlap with above.                                                                                                 |
| Biofutur                                                        | Biofutur<br>56 rue de l'Université<br>75007 Paris<br>France                                             | 11               | FF315 (France),<br>FF355 (UK and USA)          | As usual the French have come up with the only touch of class. Glossy magazine — rather general treatment with reviews and not much up-to-the-minute news. Good reading, has a thoughtful approach, and improves your French.                         |
| Biomass Bulletin                                                | Multi-Science Publishing Co. Ltd<br>42/45 New Broad St<br>London EC2M 1QY<br>UK                         | 4                | £48, \$96                                      | Covers all aspects of fuel from biomass, recycling. All journals, no patents. Straightforward abstracts — no hard sell. Includes annual index.                                                                                                        |
| Biomass Digest                                                  | Technical Insights Inc.<br>158 Linwood Plaza<br>PO Box 1304<br>Fort Lee<br>NJ 07024<br>USA              | 12               | \$168 (North America),<br>\$192 (elsewhere)    | Reports on journal articles, patents, meetings and gossip. Also includes editorial statements from guest authors (who are identified).                                                                                                                |
| Biotech News                                                    | Microinfo Ltd<br>PO Box 3<br>Alton<br>Hampshire GU34 2PG<br>UK                                          | 12               | £65 (UK),<br>£70 (elsewhere)                   | Reports more UK events than most. Tries to provide some background to events and often gives source of information.                                                                                                                                   |
| Biotechnology Abstracts (Derwent Biotechnology Abstracts)       | Derwent Publications Ltd<br>Rochdale House<br>128 Theobalds Rd<br>London WC1X 8RP<br>UK                 | 24               | £275 (£375 for 18 months if paid by Jan. 1983) | No-nonsense abstracts of all literature (including patents) in all areas of biotechnology. No news, no gossip, no opinions, just straight reporting of primary source material.                                                                       |
| Biotechnology Bulletin                                          | Scientific & Technical Studies<br>Bath House (3rd Floor)<br>56 Holborn Viaduct<br>London EC1A 2EX<br>UK | 12               | £80                                            | Covers all areas of biotechnology from all sources, but strong on gossip with no source indicated, even when this could be helpful; e.g. "Intervet International markets first DNA vaccines". Promises much but doesn't deliver a lot.                |
| Biotechnology Bulletin Reports                                  | Scientific & Technical Studies<br>(see Biotechnology Bulletin)                                          | 12               | £48                                            | Companion to <i>Biotechnology Bulletin</i> with reports on laboratories, companies or government departments. Provides contact names.                                                                                                                 |
| Biotechnology Information                                       | Paul Mayes<br>Library Services<br>Teesside Polytechnic<br>Middlesbrough<br>Cleveland TS1 3BA<br>UK      | 12               | Free                                           | Title listing only — a local service, not very useful compared to others, but free. Can't cover everything and doesn't.                                                                                                                               |
| Biotechnology Law Report                                        | Mary Ann Liebert (see Agricultural Genetics Report)                                                     | 12               | \$275 (North America),<br>\$299 (elsewhere)    | Analysis of past, present, and future biotechnology litigation. Has a large editorial board of legal and patent experts.                                                                                                                              |
| Biotechnology News                                              | CTB International Publishing Co.<br>PO Box 579<br>Summit<br>NJ 07901<br>USA                             | 26               | \$185 (USA),<br>\$210 (elsewhere)              | One of the better "me-too" publications in being cheaper and more frequent. Lacks good titles to attract attention. Printed on yellow paper, but not yellow journalism.                                                                               |
| Biotechnology Newswatch (McGraw-Hill's Biotechnology Newswatch) | McGraw-Hill Inc.<br>1221 Avenue of the Americas<br>New York<br>NY 10020<br>USA                          | 24               | \$377                                          | Covers relatively little in each fortnightly issue but does it in detail. Tries to provide information behind biotechnology news, not just the headlines. More extensive coverage geographically than most.                                           |

| Publication                  | Publisher                                                                                 | Issues per annum | Subscription rate per annum                                                  | Comments                                                                                                                                                                                                                              |
|------------------------------|-------------------------------------------------------------------------------------------|------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Biotechnology Patent Digest  | OMEC Publishing Company<br>PO Box 546<br>Great Falls<br>VA 22066-0546<br>USA              | 26               | \$235 (North America),<br>\$265 (elsewhere)                                  | Reads the patent office output for you. Appears to use patents of the past (nineteenth, early twentieth century) as filler. Expensive history with little relevance. Has cheap(er) personal subscription rate.                        |
| Biotechnology Press Digest   | Mary Ann Liebert (see Agricultural Genetics Report)                                       | 12               | \$185 (USA), \$217 (elsewhere)                                               | Mostly press clippings with very limited primary source information. Thus this publication provides the best of the gossip and probably the least of the facts.                                                                       |
| Biotech Quarterly            | Science & Technology Letters<br>12 Clarence Rd<br>Kew<br>Surrey TW9 3NL<br>UK             | 4                | £12                                                                          | The official bulletin of the British Coordinating Committee for Biotechnology. More of a brief magazine — has articles, book reviews, education reviews. Tends to be more political (mostly UK) in content.                           |
| Biotech-Update               | Scientific Newsletters Inc.<br>PO Box 4546<br>Anaheim<br>CA 92803<br>USA                  | 10               | \$95 (North America),<br>\$105 (elsewhere)                                   | "Biotechnology as it relates to the clinical laboratory". Discusses books, meetings, products and journal articles. Limited information and virtually no analysis. Not good value if reader wants news.                               |
| Frontiers in Immunoassay     | Scientific Newsletters (see Biotech-Update)                                               | 10               | \$45 (North America),<br>\$51 (elsewhere)                                    | Reports all information on assay methods for "laboratorians". Some news, some reviews, but mostly lists of references, books etc. Same publisher, same format as <i>Biotech-Update</i> .                                              |
| Genetic Engineering Letter   | Environews Inc.<br>1097 National Press Building<br>Washington DC 20045<br>USA             | 24               | \$295                                                                        | One of the first, and almost the worst. Genuine (awful) parish newsletter production. Newsy reporting of a few key items in each issue — looking for the hot topics.                                                                  |
| Genetic Engineering News     | Mary Ann Liebert (see Agricultural Genetics Report)                                       | 6                | \$90 (USA), \$106 (elsewhere)                                                | Good coverage of major items — even includes photographs — and is the most newsy publication of the lot. Has advertisements, equipment reports. A true newspaper for the field, headlines and authors. Lively, but only a bi-monthly. |
| Genetic Technology News      | Technical Insights (see Biomass Digest)                                                   | 12               | \$180 (North America),<br>\$204 (elsewhere)                                  | Another "me-too" production. Nothing editorially to distinguish it from <i>Biotechnology News</i> or <i>Genetic Engineering Letter</i> . All come from the same mould.                                                                |
| Industrial Biotechnology     | Industrial Biotechnology<br>5th Floor<br>31-33 High Holborn<br>London WC1V 6BD<br>UK      | 12               | £115 (UK), £130 (elsewhere)                                                  | Monthly with British interests foremost. Emphasis on review articles with illustrations — presents overviews rather than what's new. Professional looking and nicely produced on fetching pale blue paper.                            |
| Practical Biotechnology      | Practical Biotechnology<br>4 Woodlands<br>Harpenden<br>Herts<br>UK                        | 6                | £90 (UK), \$200 (USA)                                                        | British, more sober version of <i>Genetic Engineering News</i> in bulletin format (but without advertising or photographs). Well written and produced on glossy paper.                                                                |
| Radioassay-Ligand Assay News | Scientific Newsletters (see Biotech-Update)                                               | 10               | \$65 (North America),<br>\$75 (elsewhere)                                    | From the same stable as <i>Frontiers in Immunoassay</i> — since the two cover the same general area of interest why not publish them as one?                                                                                          |
| Scrip                        | PJB Publications Ltd<br>18/20 Hill Rise<br>Richmond<br>Surrey TW10 6UA<br>UK              | 104              | £195 (UK), £235 (Europe, Middle East, North Africa),<br>£249 (rest of world) | Has presented pharmaceutical news for some time — has good following even though not always accurate. Doesn't emphasize biotechnology but covers pharmaceutical and other fields as whole.                                            |
| Telegen Reporter             | Environment Information Center Inc.<br>48 West 38th Street<br>New York<br>NY 10018<br>USA | 12               | \$895                                                                        | Has the most coverage, most services offered and justifiably the most expensive. May not come out often enough to catch information when it is fresh.                                                                                 |

Omitted are *BioEngineering News* and *D-J-M Enzyme Report* (published by Deborah J. Mysiewicz Publishers Inc., Suite 304, 109 Minna St, San Francisco, CA 94105, USA) which were not submitted for review.

\*This information is accurate as far as is known, but before subscribing readers should check with the publishers concerned.



# Trooping the harem

F.J. Bollum

**Molecular and Cellular Biology.** Editor-in-chief Aaron J. Shatkin. 12/yr. (American Society for Microbiology.) \$84. **Comments on Molecular and Cellular Biophysics.** Coordinators Burt V. Bronk and J.W. Longworth. 6/yr. (Gordon & Breach.) \$114, £63. **Bioscience Reports.** Chairman editorial board C.A. Pasternak. 12/yr. (Biochemical Society, PO Box 32, Colchester, UK.) £95, \$215. **Biochemistry International.** Editor-in-chief A.W. Linnane. 12/yr in 2 vols. (Academic.) £121.60, \$225. **The Journal of Biosciences.** (Indian Academy of Sciences.) Subscription rate not known.

WHEN it comes to innovation in publishing, Johann Gutenberg did it all. Many scientific publishers have reduced the time lag between manuscript submission and hard copy circulation from Gutenberg's 15-year schedule, but the importance of the time factor is mostly in the eyes of certain beholders and not all beholders are looking at the same end of the elephant. An old friend of mine feels that those with important things to say should be allowed to chisel their words on stone tablets and carry them to the library! This crop of new journals in biochemistry and molecular biology provides time-tested solutions to these age-old arguments, some with better style than others.

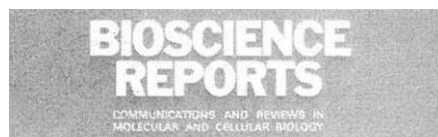
**Molecular and Cellular Biology (MCB)** is a hard-science, hard-print, hard-reviewed journal sponsored by the American Society for Microbiology. It has a formidable list of editors to ensure quality and the science is the eukaryotic molecular biology of microbial and higher organisms. Established journals with similar words in their titles seem more pretentious (*Cell*), more stodgy (*Journal Molecular Biology*)

## Molecular and Cellular Biology

or too classical (*Journal Cell Biology*). Publication time in MCB sets no new records, but reasonable price — especially low (\$18) for ASM members — and high overall quality make it an outstanding new publication.

**Comments on Molecular and Cellular Biophysics (CMCB)** is of interest for quite different reasons. This journal proposes to publish the thoughts of working scientists on subjects ranging from molecular motions to the social fallout from technology. Topics appear to be unrestricted, wide ranging and open-ended. Thus, Rosalyn Yalow disputes the interpretations of K.Z. Morgan on the risks of low-level radiation, published in a previous issue. Coordinators (editors) add footnotes to explain unusual English

usage, reviewers' questions are addressed by the author at the end of some papers and letters from readers in the "Comments Roundtable" round out the lively interchange. Brief conference reports are also included. The articles seem to emanate



from working scientists, not scientific journalists, and the views expressed appear to originate from the personal experience of scientific life.

Scientists are frequently criticized for their inability to communicate. But the rigidity of standard scientific publications puts severe limits on that. And what a thinking scientist has to say may appear too technical or limited in scope for the *Times* or *Post* space commitments. *Discover*, *Science* '82 and similar magazines written by journalists for lay consumption are based on limited interviews with designated scientists. Perhaps CMCB (which has companion publications covering inorganic chemistry and geochemistry) will provide new source material for scientific journalists and encourage scientists at this level of communication. The journal is "coordinated" by Burt Bronk and John Longworth with an international list of correspondents; price is fairly stiff, but the performance here is enticing.

Several members of the troupe seem less attractive, having off-colour complexions, questionable credentials, missing fangs or show signs of dissipation. **Bioscience Reports** is sponsored by the Biochemical Society in the UK and claims to publish short papers and reviews in molecular and cellular biology. It is stated that publication will be rapid and the articles of high quality. However, I fail to see the urgency of Nobel Laureate autobiographies or most of the other papers published in the issues I reviewed. The fangs are missing, but if you are tired and want to reminisce you will probably find this trouper at the rear of the caravan.

**Biochemistry International (BI)** is an Academic Press expansion into down-under rapid publication of technical papers. Published under the aegis of the International Union of Biochemistry, BI has the format and content of *Biochemical and Biophysical Research Communications*. Papers are reviewed and publication is rapid — manuscripts received October 20 (from Australia) are published in the November issue. The international flavour is reflected by papers from Moscow State (USSR), Montana State (USA), Valdivia (Chile), Nigeria, India, Japan, Taiwan and so on. This is a well-worn performance.

You pay your money and you get your pick.

**The Journal of Biosciences**, formerly the experimental biology section of the *Proceedings of the Indian Academy of Sciences*, publishes original papers of rather uneven quality in a variety of biological disciplines. The complexion of the journal, hard print on pinkish paper, is unusual. At \$10 per year, it is selling below the cost of postage. Both this publication and BI do fill a need for Southern Hemisphere and Australasian scientists. I expect that these journals should be available in your institutional library.

Novelist-playwright Brendan Behan compared book critics to eunuchs in a harem; knowing everything of the craft but incapable of the performance. In the scientific publishers' caravan, new journals are the harem troupe, subject to the whims of fellow-travelling authors, editors and readers. Some of this year's selections will be next year's dung sweepers.

F.J. Bollum is a Professor of Biochemistry at the Uniformed Services University of the Health Sciences, Bethesda, Maryland.

# Impure thoughts

Christopher R. Lowe

**Applied Biochemistry and Biotechnology.** Executive editor H.H. Weetall. 6/yr. (Humana.) \$125 US, \$132.50 elsewhere. **Journal of Applied Biochemistry.** Editor-in-chief P.N. Campbell. 6/yr. (Academic.) £39, \$60.

It is apparent that the continued development of biotechnology will be contingent not only on advances in basic biological science and the technology of handling biological materials, but also on the efforts of those lateral thinkers able to apply such basic knowledge across the boundaries of established scientific areas. Not surprisingly, therefore, journals which bridge the gap between the underlying pure science of several disciplines and the more applied aspects are essential ingredients in the healthy growth of biotechnological enterprise.

Both the *Journal of Applied Biochemistry (JAB)* and *Applied Biochemistry and Biotechnology (ABB)* are broad-spectrum journals dedicated to presenting innovative concepts in the emergent and highly interdisciplinary field of applied biochemistry. To my mind, ABB (formerly the *Journal of Solid-Phase Biochemistry*) fulfils more nearly the promises of its editorial manifesto; the journal contains a veritable treasure-trove of novel ideas that would ignite the imagination of even the most languid post-graduate. Its scientific

# Selected new journals from Karger

## Complement

A consolidated view of new work on the complement system and its role in various immunologic responses.

Editor: U.E. Nydegger, Bern  
1983: Volume 1 with 4 issues  
Institutional subscription: SFr. 196.-

## Developmental Pharmacology and Therapeutics

Clinical studies, animal research relevant to general developmental pharmacology, preliminary clinical and laboratory observations, and reviews of recent advances in perinatal and pediatric drug therapy.

Editor: J.V. Aranda, Montreal  
1983: Volume 6 with 6 issues  
Institutional subscription: SFr. 220.-

## Invasion and Metastasis

Cancer research, providing maximum exposure for research on cancer dissemination and tumor cell heterogeneity.

Editors: J.C. Salomon; B. Sordat  
1983: Volume 3 with 4 issues  
Institutional subscription: SFr. 180.-

## Magnesium

Experimental and clinical research covering all aspects of magnesium in health and disease, including findings from biochemical and nutrition studies.

Editor: B.M. Altura, Brooklyn, N.Y.  
1983: Volume 2 with 4 issues  
Institutional subscription: SFr. 204.-

## Neuroepidemiology

A reference to progress achieved when epidemiologic methods are applied to acquire new understanding of clinically relevant problems in neurology.

Editor: B.S. Schoenberg, Bethesda, Md.  
1983: Volume 2 with 4 issues  
Institutional subscription: SFr. 140.-

## Stem Cells

Experimental and clinical work concerned with normal and neoplastic stem cell differentiation and proliferation.

Editor: M.J. Murphy, Jr., Dayton, Ohio  
1983: Volume 3 with 4 issues  
Institutional subscription: SFr. 172.-

## Survey of Immunologic Research

Guidance through the vast literature of immunology provided in succinct expert review articles.

Editor: J.M. Cruse, Jackson, Miss.  
1983: Volume 2 with 4 issues  
Institutional subscription: SFr. 228.-

Free sample copies available on request  
Circle No. 38 on Reader Service Card.

S. Karger AG  
P.O. Box  
CH-4009 Basel  
Switzerland



content is presented in an attractive and lucid style and includes, in addition to original research articles, topical reviews, literature, patent and book reviews, lists of market studies describing the commercial opportunities in biotechnology, announcements of forthcoming events and an interesting little section on general news and gossip.

The principal editors of ABB are to be commended for imposing a great degree of cohesion upon what is potentially a wilderness of diverse interests, and for including all the supplementary paraphernalia which is undoubtedly a feature of modern applied biochemistry.

JAB is less to my liking. Recently, I'm not sure when, the sub-title "With Reference to Biotechnology" was added to the journal's handle which would seem to indicate a case of clinging to the bandwagon by the fingertips. The journal appears to stretch its declared editorial policy by including a mélange of papers, some rather mundane, others more suited to established journals of pure science. Indeed, the issues I perused contained a surprisingly large proportion of papers, particularly those with a biomedical flavour, which were not obviously

results of fundamental studies directly related to the design of new biotechnology, the improvement of existing biochemical processes and the preparation, utilisation, conversion or application of biological materials.

There is a danger in too loose an interpretation of such an editorial policy, in that there can be loss of direction and the journal becomes merely a repository for papers which could just as easily be dispersed amongst other journals. However, in all fairness, JAB does contain some front-line papers from established



applied scientists and, unlike ABB, has a mechanism for the early publication of results in short communication format.

Clearly, these two publications occupy an important niche between the established journals of pure biochemistry and those covering the more "spanner and pipe-work" side of bioengineering (although for journals dedicated to the publication of innovative research, their covers make even an obituary page look inviting). Both could be improved by more aggressive packaging to attract readers, subscribers and potential authors alike, for without the latter, voting with their pens, no journal can survive.

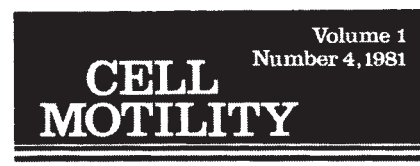
Christopher Lowe is a Lecturer in Biochemistry at the University of Southampton.

# Cells in motion

Jeremy Hyams

*Cell Motility*. Editor R.D. Allen. 6/yr.  
(Alan R. Liss.) \$105 US, \$121.50 Europe.

THE STUDY of cell movement in its many different forms is now a major part of cell biology, generating several thousand papers each year. With these spread widely throughout the existing literature there was obviously a need for a new journal to provide a central forum for research in this area. The *Journal of Muscle Research and Cell Motility* (reviewed in *Nature* 293, 344; 1981) made an attempt to fill this role but in practice has remained very much specialized towards muscle. *Cell Motility* on the other hand is a broadly based publication and already seems to have caught the imagination of workers in the field.



The journal was introduced as a quarterly but now appears bimonthly. It promises rapid publication, although whether it lives up to this boast is difficult to judge as articles are not dated. If it is in fact the case, then speed is not achieved at the expense of quality — overall production is good and the reproduction of micrographs, so important in this type of work, is excellent.

The most encouraging feature of this new journal, however, is the composition of the editorial board which, unlike many American journals these days, acknowledges that interesting research goes on outside the United States. It is particularly appropriate that Japan, which produces an increasing amount of innovative work on motility is well represented.

As well as being international in scope the editorial board is catholic in its interests. This is reflected in the first two volumes which have covered a variety of topics ranging from a cytological description of chromosome movement in crane fly spermatocytes to the identification of actin and myosin in tomatoes! The journal also publishes the occasional invited review and, most usefully in times when travel money is scarce, reports of international meetings.

All in all this is a valuable addition to the scientific literature. It will be a great pity if the limitations on spending now being imposed on scientific libraries prevent *Cell Motility* becoming widely available.

Jeremy Hyams is a Lecturer in the Department of Botany and Microbiology at University College London.

# The ascent of andrology

R.J. Aitken & G.D. Chisholm

*Journal of Andrology*. Editor A. Bartke. 6/yr. (Lippincott.) \$49 US, \$57 elsewhere (personal); \$57 US, \$66 elsewhere (institutional). *International Journal of Andrology*. Editor N.E. Skakkebaek. 6/yr. (Scriptor.) Dkr. 500. *The Prostate*. Editors-in-chief A.A. Sandberg and G.P. Murphy. 6/yr. (Alan R. Liss.) \$120 US, \$136.50 Europe.

IT IS A curious fact that while the study of female reproduction has flourished during the past 20 years, giving rise to revolutionary new methods of either preventing or inducing conception, the study of male reproduction has remained in its infancy. A movement towards redress of this imbalance has gained impetus over the past few years and, in this light, the appearance of new andrological journals is to be welcomed.

The function of publications such as the *International Journal of Andrology* (IJA) and the *Journal of Andrology* (JA) should be to open up channels of communication between scientists and clinicians sharing a common interest in male reproduction. In this respect, these two journals succeed. Both of them cover similar ground, publishing clinically orientated papers on the diagnosis and treatment of male infertility, and reports dealing with the fundamental mechanisms of male reproduction in animal models.

The papers in JA are of good scientific quality and the standards of production, including micrographs, are high. Most of the material published in this journal originates from laboratories in the United States and includes abstracts of meetings of the American Society of Andrology (of which it is the official organ). Such abstracts are a useful way of assessing the direction of research in States-side laboratories without waiting for the publication of full papers, which in this journal takes around nine months.

The IJA is a European-based journal which has been in production since 1978, although the helm has recently been taken by a new editor. He inherits a healthy ship which, with a slight tightening of the editorial grip, will prove a ready match for its American counterpart.

These two journals are not the only ones covering andrology to have appeared in recent years, but they are the best. Both need to reduce publication times in order to keep abreast of developments; but, this achieved, they should make a significant contribution to the science and clinical practice of andrology.

The editors of *The Prostate* felt that the time spent in finding the relevant literature on this gland (and other male accessory sex glands) was now such a chore that the time had come to collect related papers into a

single, organ-specific publication. It appears that others agree; at first the journal appeared quarterly, but now bimonthly.

The editors are based at the Roswell Park Memorial Institute, Buffalo, and are closely linked with the activities of the US National Prostatic Cancer Project (NPCP). Thus it is no surprise to find that most of the contents of the first three volumes are derived from these sources. This has ensured a high level of investigative input and also a dominance of North American contributors. However, there is an increasing international character, both in the original articles and in the reporting of meetings which have included abstracts of conferences of the European Society for Urological Oncology and Endocrinology.

Because much of the material represents the activities of the NPCP, there is a good proportion of human prostate as well as rat prostate-related work: in Vol. 3, for

example, half of the papers are either clinical studies or reports using human prostatic tissue. All articles appear as original papers, but some of these are updated reports, some are parts of a major study, and some are entirely new work. This blend is an advantage, for the journal is also a useful reference source and for this alone has much value. There are no reviews and no correspondence columns.

Whether or not this journal can attract important new material away from the more established international journals remains to be seen. Also, it is doubtful if a

## The Prostate

clinician will derive much benefit from *The Prostate* for the clinical journals are now so numerous as to defy more than fast scanning. Nonetheless the journal is good value, it fills a gap in the field and the commitment to the subject shown by the editors should ensure a good future. □

R.J. Aitken is in the MRC Reproductive Biology Unit, Edinburgh, and G.D. Chisholm in the Department of Surgery at the University of Edinburgh Medical School.

## Variations for clinicians

Hilary Pickles

*Clinical Physiology*. Editor J. Wahren. 6/yr. (Blackwell Scientific.) £67.50 UK, \$180 US, £81 elsewhere. *Journal of Cerebral Blood Flow and Metabolism*. Editor A.M. Harper. 4/yr. (Raven.) \$89 US, \$99 elsewhere (personal); \$105 US, \$115 elsewhere (institutional). *International Journal of Clinical Pharmacology Research*. Editor A. Bertelli. 6/yr. (Bioscience Ediprint.) \$136. *Pediatric Pharmacology*. Editor S.J. Yaffe. 4/yr. (Alan R. Liss.) \$75 US, \$86 Europe. *Developmental Pharmacology and Therapeutics*. Editor J.V. Aranda. 8/yr in 2 vols. (Karger.) SwFr. 154, \$83 (personal); SwFr. 220, \$119 (institutional). *The Clinical Biochemist: Reviews*. Editors I. Farrance and M.J. Staley. 3/yr. (Australian Association of Clinical Biochemists.) A\$20.

*Clinical Physiology* (CP) is published for the Scandinavian Society of Clinical Physiology and is edited from the renowned department at the Karolinska. Although reports on animal experiments relevant to human physiology and pathophysiology are acceptable, the great majority of papers refer to human experimental research using patients or volunteers. Publication delay appears to run at about nine months, but a substantial proportion of this appears to be taken up by the refereeing process.

In all, this is an excellent journal. The quality of the papers and style of production are hard to fault, and the standard of English is impeccable; it is hard to believe that for most authors this is not their first language. It is witness to the strength of the Scandinavians in this field that they are able to fill six issues a year, averaging nine full papers each, with little help from the rest of the world. Academic departments of clinical physiology are less common outside Scandinavia, however, and it remains to be seen if the journal can attract appropriate papers (and subscriptions) from elsewhere. A journal of this quality is surer of success than most, and CP deserves to become established as an international publication of high standing.

Another impressive journal is the *Journal of Cerebral Blood Flow and Metabolism* (JCBF). With the development of new techniques in this important area, research output had grown apace and, as explained in the opening issue, was being disseminated in a very wide range of journals. To simplify matters, a new society — the International Society of Cerebral Blood Flow and Metabolism — was formed to go with the new journal.

Alerting workers to the existence of a new publication is always a problem, as many researchers are loyal to journals in which they have already published. The



editors of JCBF overcame this problem by putting together an enormous supplement to the first volume containing over 300 abstracts of a major international meeting, thus ensuring that everyone in the field heard of the journal. The journal itself carries both original research papers and review articles, and the scientific standard and quality of presentation is high. The review articles in particular should prove indispensable.

## Developmental Pharmacology and Therapeutics

The *International Journal of Clinical Pharmacology Research* is also a society journal, being published for the Italian Society of Clinical Pharmacology, but there parallels with the preceding two journals end. The quality of the papers does not match the eminence of the multinational editorial board, and there is little evidence of a refereeing process at work. Many papers are padded out with irrelevant detail and contain a minimum of original data; in addition there are frequent departures from the published instructions to authors.

Many authors might with advantage take note that several European languages are acceptable, and English is only preferred, not required. Frequent Italianisms are found — "plasmatic reninic activity", "sinusal rhythm", "heroine intoxication" — and many papers are merely intelligible, not fluent. There are no hints to the publication delay, but it took two years for some conference proceedings to be published in the first issue. At present two-thirds of the papers come from Italy, and the standards will need to be raised before this journal lives up to the "International" in its title.

After disasters with chloramphenicol and thalidomide, it became appreciated that the developing organism may not respond to drugs in the same way as the fully grown adult. The first issues of both *Pediatric Pharmacology* (PP) and *Developmental Pharmacology and Therapeutics* (DPT) carry editorials justifying this growing speciality and the issue of a new journal, DPT supporting its argument with an analysis of the journals that had previously carried material on pharmacology in the developing animal. So instead of 100+ journals, there are now 102+ for researchers to scan.

The material carried by these rival publications is very similar, being almost equally divided between animal and human studies and the great majority of papers coming from North America. Although papers are said to be subject to editorial review, it appears as if neither journal is able to be as selective as it would have liked; for instance, DPT seems to allow case reports to be presented as full original papers. Neither journal gives information on

publication delay, and both appear to have been struggling — PP produced only half the issues it had promised in 1980–1981 and DPT, although now managing eight issues per year rather than the six it started with, has, according to *Current Contents*, been months late with its recent issues.

Finally, *The Clinical Biochemist: Reviews* is a different type of journal, and unlike the others is not listed in *Current Contents*. It is published for the Australian Association of Clinical Biochemists and its main purpose is postgraduate education, with hints on passing postgraduate exams. As well as review articles it has a bibliography series, where the key papers in a field are listed and briefly reviewed. Interestingly, for most articles the name of the referee is given almost equal prominence to that of the author. Although written by Australians for their own consumption, it could prove helpful to chemical pathologists elsewhere, particularly those studying for higher exams. □

Hilary Pickles is a Senior Medical Officer in the Medicines Division of the Department of Health and Society Security, Vauxhall, London.

## Viral treatment

B.W.J. Mahy

*Antiviral Research*. Executive editors A. Billiau and E. De Clercq. 6/yr. (Elsevier Biomedical.) Dfl.220, \$88. *Journal of Interferon Research*. Editor William E. Stewart II. 4/yr. (Mary Ann Liebert, New York.) \$65 US, \$81 elsewhere (personal); \$135 US, \$151 elsewhere (institutional).

THE possibility that virus diseases might be controlled by drugs or treatment with interferon has been actively explored for the past 25 years. But although some antiviral drugs such as idoxuridine found their way into clinical use, treatment was confined to topical application because of their non-specific nature and associated side-effects. The advent of acycloguanosine in 1977 showed that specific, non-toxic treatment of some herpes virus infections was possible, and revived interest in antiviral chemotherapy. Interferon, too, remained a fascinating but highly esoteric subject for research until the recent application of biotechnology revealed the existence of multi-gene families of interferons with implications in many branches of biological science.

It is no surprise that these developments have caught the interest of publishers as well as of scientists. Other than the five established virology journals (*Archives of Virology*, *Intervirology*, *Journal of General Virology*, *Journal of*

*Virology* and *Virology*), the only outlet for papers in these areas has been provided by two antimicrobial journals which were started in the mid-1970s, namely *Antimicrobial Agents and Chemotherapy* and *The Journal of Antimicrobial Chemotherapy*. Neither journal has provided the right focus for antiviral papers, however, as their main emphasis has been on antibiotic studies.

*Antiviral Research* (AR) was launched from the Rega Institute in Belgium with a wide coverage, including not only antiviral chemotherapy and interferon research but also the development and clinical assessment of virus vaccines, and cellular and humoral immunity to virus infections. The latter areas are well represented in a number of immunological journals, but no doubt the publishers felt it wise that their infant should have as broad a base as possible.

Most of the papers so far published have dealt with antiviral drugs or interferons, but several useful papers and review articles on cellular immunity mechanisms have also appeared. However the journal has not yet attracted the sort of key papers on antiviral drugs and their mechanisms of action for which it should ultimately become the forum: these still appear in more general science journals. AR is nonetheless well produced and has some distinguished scientists associated with the editorial board; it can be recommended as a journal likely to survive and find a permanent home on medical library shelves.

The *Journal of Interferon Research* (JIR) has more limited appeal. It was launched in the belief that it would provide "the primary source for original publications on all the varied aspects of interferon research". Apparently some interferon papers had received little understanding from the better established journals. The large editorial board includes seven members who also serve on the board of AR; the contentious nature of interferon research is illustrated by the instruction that "authors will be allowed to indicate one reviewer who should be excluded from review of their manuscript".

After a faltering start with only one executive editor, the editorial board have recently elected seven section editors from among their number, which should speed up the reviewing process. But the papers published so far lack general scientific interest, and it is difficult to envisage much immediate improvement in this respect. It is also a pity that the paper on which the journal is printed is of poor quality, making for problems in the interpretation of half-tones. It is hard to escape the conclusion that the JIR has too slender a base to support itself for long in the hard economic world of publishing. □

B.W.J. Mahy is Huddersfield Lecturer in Special Pathology (Virology) at the University of Cambridge.

# More environmental spread

Peter Brookes

*Mutation Research Letters*. Editor S.H. Sobels. 12/yr in 3 vols. (Elsevier Biomedical.) Dfl.638, \$255. *Teratogenesis, Carcinogenesis, and Mutagenesis*. Editor Marvin S. Legator. 6/yr. (Alan R. Liss.) \$95 US, \$111.50 Europe.

BOTH of these journals (MRL and TCM, respectively) owe their existence to the current concern that environmental chemicals may be responsible for some human cancers and certain heritable genetic disorders. Professor Hermann Druckrey foresaw this possibility when he suggested in 1972 that the word "genotoxic" should be used to describe the toxic, lethal and heritable effects on karyotic and extrakaryotic genetic material which may arise from chemical-DNA interactions.

The two journals overlap considerably in their fields of interest and are equally well produced; in particular the glossy paper allows excellent reproduction of black-and-white photographs. Also, despite clearly stated editorial policy on the subject, the journals share a lack of consistency in their presentation of references which some readers might find irritating and suggestive of weakness in the editorial office. These similarities apart, the two offer the potential author and reader quite different forms of scientific publication.

MRL is the fifth and latest addition to the sub-sections of the journal *Mutation Research*, joining the existing *Regular*, *Environmental Mutagenesis*, *Reviews in Genetic Toxicology* and *General Toxicology Testing* sections. The 1982 schedule envisages the publication of three volumes (a total of 11 issues) of MRL, in addition to the 12 volumes (32 issues) of the other sections of *Mutation Research* — the cost of the complete journal is now approaching a stiff \$1,000. In addition, I wonder if I am alone in my concern over the practice of publishers in designating a single issue of a journal as, for example, Vol. 104/4-5 so that the final issue of each volume can be given the number 6. Does this really persuade librarians that they are getting value for money?

MRL publishes material in all the areas covered by its sister-sections of *Mutation Research* but restricts its authors to a maximum of six printed pages. The initial aim was to publish full papers (certainly not brief communications as the title might imply) within three months of submission and it was hoped to inform authors within two weeks of the acceptance or rejection of their paper. This schedule would seem to require an editorial decision or very cooperative referees and appears to have been a little optimistic; based on the acceptance dates on papers in recent issues

it is proving difficult to achieve publication in less than 4-6 months. However, the format seems to be popular as judged by the increasing number of papers published each year.

The prime objective of TCM "is to foster interactions between investigators in the fields of teratogenesis, carcinogenesis and mutagenesis". The journal publishes full-length papers covering this wide area of research. No submission or acceptance date is given for the contributions.

TCM acknowledges support from the March of Dimes Birth Defects Foundation and this organization's annual conference seems to have yielded a number of papers for the journal, although this is not actually stated. The first volume of four issues appeared in 1980 while the second volume, originally scheduled for 1981, was not in fact published until 1982.

Titles of contributions in the area of carcinogenesis and mutagenesis suggest that they might equally well have been submitted to any one of many established journals. Authors of papers on teratogenesis would have a more limited choice, however, and the future of TCM may depend on its ability to attract papers in this latter area. If it can do this, and fulfil the hope of its editor and encourage collaboration between workers in its three fields of interest, it could serve a very useful purpose. □

*Peter Brookes is Chairman of the Chemical Carcinogenesis Section at the Pollards Wood Laboratory of the Institute of Cancer Research, Chalfont St Giles, Buckinghamshire.*

## Red 'flu — and other viruses

D.H. Watson

*Soviet Progress in Virology*. Editor V.M. Zhandov. 6/yr. (Allerton.) \$250 US, \$280 elsewhere.

JUST over a half century after the pioneering observations of Iwanowski on tobacco mosaic, the Institute of Virology bearing his name was founded in Moscow. This led later to the appearance of the journal *Voprosy Virusologii (Problems of Virology)* in 1956. The twenty-fifth anniversary of this publication was marked by the appearance of a special number late in 1980 and this issue was selected to be the first in a regular series of cover to cover

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English translations of the journal, published under the title *Soviet Progress in Virology*.

While Russian papers have appeared in all the world's major virological journals, these form but a small proportion of the national output. The translated version is therefore very welcome in its dissemination of Russian experimental work in the field.

Perhaps the most notable feature of the papers reproduced is their brevity and this alone means that they may be studied with profit by authors elsewhere in the world. Papers on influenza viruses predominate with Zhdanov, the editor-in-chief of the journal, and Bukrinskaya being frequent authors. One paper, on the analysis of antigenic variation of the haemagglutinins of H3N2 viruses, using monoclonal antibodies, has J. Skehel as a co-author. Papers on interferon and on the biophysical properties of bacteriophages (Tikhonenko) are also well represented. A valuable feature is the inclusion in each issue of survey articles and a section "Aids to Virologists" describing new techniques.

Most virologists will find material to interest them in successive issues of this publication — even if it must be admitted that the standard is somewhat variable — and will welcome the opportunity of easy access to current Russian work on viruses. □

*D.H. Watson is Professor of General Microbiology and Head of the Department of Microbiology at the University of Leeds.*

## Bioagrimedigen

Stephen Oliver

*Journal of Molecular and Applied Genetics*. Editor H.M. Goodman. 6/yr. (Raven.) \$80 US, \$90 elsewhere (personal); \$135 US, \$145 elsewhere (institutional).

THE *Journal of Molecular and Applied Genetics* (JMAAG) is designed to find a place in the library of every self-respecting genetic engineering company. The editors aim to publish full-length papers on the use of modern genetic techniques to solve basic and applied problems in biochemistry, medicine and agriculture. The standard of papers is high and they are well presented in large format and on glossy paper. There are no restrictions on the length of contributions and it is intended that there should be full documentation of data, although to date a number of papers with rather perfunctory methods sections have appeared.

The journal has a distinguished editorial board in which plant molecular biologists are well represented. Each issue appears without a publication date but the submission and acceptance dates of the papers are recorded; the interval between them has lengthened from less than six weeks in the early issues to about three

months. This increase reflects the fact that the journal is attracting papers from a wider circle of contributors. The majority of the papers deal with the genetics of mammals (including human beings) and plants. Bacteria, particularly those of agricultural importance, are well represented but there has been little on the microbial eukaryotes. The "applied" interest of most of the papers is rather long-term and the journal contains little for the industrial geneticist.

JMAAG is a worthy journal which has, however, failed to identify a distinct subject area and is thus in competition with the likes of *Cell*, *Gene*, *Plasmid* and *Molecular and General Genetics*. It is distinguished by its high proportion of papers dealing with plant molecular biology, a subject which is not well served by the scientific press; one would have thought it better for the journal to have concentrated on this particular area of activity.

So, despite the high quality of its contents, the lack of a readily identifiable constituency combined with powerful competition means that it is difficult to predict an easy future for the journal. □

*Stephen Oliver is Senior Lecturer in Applied Molecular Biology at the University of Manchester Institute of Science and Technology.*

## New...and necessary

### The International Journal of Robotics Research

Michael Brady, MIT  
Richard Paul, Purdue University  
Editors

Volume I, 1982

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## Good food guide

John Rivers

*Appetite*. Chief editor D. Booth. 4/yr. (Academic.) £20.80, \$54.50 (personal); £41.50, \$109 (institutional).

FIRST impressions of a new journal are like those of a new house: it's somehow incomplete, empty and echoing. The first issues of *Appetite* are certainly like that — desperately filled with editorials, expansively laid-out notices, abstracts, reviews, prolix correspondence and even an inconsequential historical note on the word appetite itself. Only 40 per cent of the first issue was made up with original scientific papers, and three out of the four of these were work presented in higher degree theses.

All this is a common situation since sending work to a new journal is a great act of faith. But just as what matters about a new house is the extent to which it can be made into a home, what measures the value of a journal is how rapidly it changes from this initial state. The speed with which *Appetite* has been transformed suggests it meets a real need and predicts a likely future importance.

Thus by the beginning of Vol.3 it is filled

with the familiar furniture of scientific journals, 80 per cent of its pages being original communications of various kinds. The speed of this sea-change may relate to the breadth of those papers. In this journal a report on cynophagy (the eating of dogs) lies cheek by jowl with studies on saccharine appetite in the rat, and reports on zinc overdosing in chickens coexist happily with studies of coffee consumption in man.

I hope *Appetite* won't therefore be labelled an interdisciplinary journal, since this necessarily calls up the spectre of cynophagiology as an academic subject. But its eclectic nature and its unique role as a common meeting ground for scholars interested in all aspects of the consumption of food, does deserve recognition. I only hope its lack of a clear, conventional subject affiliation will help rather than hinder its acceptance by the other side of the journal partnership — the library-purchasing committee. I at any rate will recommend it to our committee most highly. Because it is not only a good journal; provided its editors don't succumb to an incipient tendency to jargon it will remain a damn good read. □

*John Rivers is a Lecturer in the Department of Human Nutrition at the London School of Hygiene and Tropical Medicine.*



# More, more and more on peptides

Piers Emson

**Regulatory Peptides.** Editors-in-chief S.R. Bloom and F.E. Bloom. 12/yr in 3 vols. (Elsevier Biomedical.) Dfl. 630. **Neuropeptides.** Editors M.J. Brownstein and J. Hughes. 6/yr in 2 vols. (Churchill Livingstone.) £44, \$93. **Peptides.** Chief editor A. Kastin. 6/yr. (Ankko International, Fayetteville, New York.) \$45 (personal); \$190 US, \$200 elsewhere (institutional).

SINCE the early 1970s, research into the physiology of the central and peripheral nervous system has been dramatically complicated by the discovery of neuro-nally-localized, biologically-active peptides. Some of these peptides were isolated in consequence of research into



hypothalamic releasing hormones or factors, notably by the groups of Guillemin at the Salk Institute and of Schally in New Orleans; others were initially discovered by Hughes and Kosterlitz at Aberdeen, as a result of the search for the endogenous ligand for the opiate receptor. Subsequently, other gut peptides, such as cholecystokinin and vasoactive intestinal polypeptide, have been found in the nervous system.

The physiological roles of these peptides are likely to be varied and they may act, for example, as conventional neurotransmitters as well as in the longer-term as hormones and trophic factors. It is the aim of the three new journals reviewed here to attract and publish original work on aspects of these burgeoning areas of research.

Of the three journals two, *Neuropeptides* and *Regulatory Peptides* (RP), are European, whilst the third, *Peptides*, is American. What is interesting about the origin of each of these publications is that they can, at least in part, be related to the three pioneering groups of the early 1970s. Thus, *Neuropeptides* is co-edited by John Hughes, and RP by Floyd Bloom at the Salk Institute, whilst Andrew Schally is on the editorial board of *Peptides*. This difference in provenance is also reflected in the initial volumes of each of the journals, as the editors have clearly sought articles from friends and colleagues.

RP is well produced and printed on a high-quality paper, which is well suited for reproduction of histochemical and anatomical figures. The editors hope to "emphasize the ability of peptides to control their targets according to some inferred rule", but it is as yet too soon to judge if they will succeed in this aim. The

articles in the first volumes cover a number of topics although a leaning towards the physiology of gut peptides is evident.

The scope of *Neuropeptides* is more restricted, the journal being concerned only with neuronal peptides. It is printed by photo off-set from the authors' text, resulting in an unattractive appearance and a lower quality, although adequate, reproduction of photomicrographs. Glancing through the early articles, *Neuropeptides* seems to have attracted more chemically-orientated papers with a bias towards chromatography and immunological characterization of neuropeptides.

Finally, *Peptides* has as its title implies a very broad brief covering any aspect of peptide research. It is well produced, and publishes high-quality photomicrographs including coloured plates. The initial articles cover a wide range of subjects but with emphasis on the behavioural effects of peptides.

Each of the journals has useful extra features and supplements; *Neuropeptides* publishes the University of Sheffield Peptide Bibliography, whilst RP and *Peptides* have included meeting supplements and abstracts. The scientific standard of articles in all three is patchy but reasonable.

I would congratulate all of the editors concerned on achieving a good standard through the difficult launch period of their respective publications. Although from the point of view of the consumer I would have preferred the appearance of just one, high-quality journal covering peptides, the rapid expansion of research in this area would seem to assure the future of all of these three newcomers.

Piers Emson is Senior Scientist at the MRC Neurochemical Pharmacology Unit, Cambridge.

## ...and tomorrow

Richard J. Hodes

**Immunology Today.** Editor J.R. Inglis. 12/yr. (Elsevier Biomedical.) Dfl. 295 + compendium.

IN A field such as immunology which is saturated with journals, it is not an easy task to create a new publication which provides a unique contribution to the literature. *Immunology Today*, however, has been successful in doing just this. Journals of reasonable quality, dedicated primarily to the refereed publication of

individual research contributions, already serve both general immunology and several of its sub-specialities. In contrast, the editorial staff of *Immunology Today* have created an unconventional format which includes several novel features.

One regular component of the publication is its "News and Features" section which is dedicated to summaries of and comments upon the proceedings of selected international meetings. In par-



ticular, emphasis has been placed upon the rapid reporting of relatively specialized meetings which are attended by small numbers of participants but which have general interest and implications. This section has to date been very valuable, the writers being well-chosen authorities who have greatly extended the audience benefiting from several important meetings.

"Compass" is a second unique feature in which critical commentaries are solicited in response to recently published papers, thus providing an unusual opportunity for constructive public response to published work. Another section entitled "Rostrum" is a forum for hypothesis and speculation, and has generally offered stimulating contributions dealing with timely and controversial topics in immunology.

In addition, reviews are published which have provided a number of concise yet highly readable summaries of contemporary topics. The contributions differ from those of more conventional review publications in that they are comprehensible by readers with widely disparate areas of expertise and yet do not compromise scientific accuracy or sophistication.

*Immunology Today* is a very untraditional journal which in the two years of its existence has provided at a relatively low cost a readable source of information and opinion covering broad areas of immunology. It must be emphasized, however, that its format will be successful only so long as the individual contributions continue to be of high quality. If this standard is sustained, *Immunology Today* will become, or rather remain, an important adjunct to the conventional immunology literature.

Richard J. Hodes is Chief of the Immunotherapy Section, Immunology Branch, at the National Cancer Institute, Bethesda.

## Promise of RI

David A. Clark

*American Journal of Reproductive Immunology*. Editor N. Gleicher. 8/yr in 2 vols. (Alan R. Liss.) \$90 US, \$112 Europe.

GROWING interest in immunology as applied to reproduction has spawned two new international journals in the past few years — first the *Journal of Reproductive Immunology* (reviewed in *Nature* 293, 357; 1981) and now the *American Journal of Reproductive Immunology* (AJRI).

The objective of AJRI is to concentrate the literature of reproductive immunology, now scattered widely, into a single publication. Many of the articles appearing in the AJRI have a clinical orientation, and thus this journal may be viewed as complementary to the *Journal of Reproductive Immunology* which focuses on the basic scientific issues in reproductive immunology. The editor promises quick reviews with notice of decision within 6 weeks of submission and rapid publication of acceptable articles. Such ideals are difficult to achieve and I calculated the median time ( $\pm$  SD) to acceptance from a sample of 19 peer-reviewed articles to be  $8.9 \pm 2.2$  weeks with a median time from submission to publication of  $7.4 \pm 1.5$  months.

The scope of the journal is unusually

broad and includes pregnancy immunology, fertility immunology, developmental immunology and "reproductive tumor immunology". Several papers in the last two categories which appeared in early issues of AJRI seemed more suited for general cellular immunology or tumour biology journals, but most papers in recent issues do appear to be more relevant.

Other features include editorials, methods papers, short communications, invited reviews and "Hypotheses". A forum for discussion is provided by letters to the editor and, in one instance, a controversial article was published together with the signed reports of referees and the authors' rebuttals — an innovation that might profitably be considered by other journals. Another unusual feature is the regular publication of a list of successful NICHD grant applicants, together with amount awarded and an abstract of the grant proposal.

The technical quality of the publication is excellent and the scientific standard of the papers, initially mediocre in some instances, has improved substantially in recent issues. AJRI has a long way to go, but the journal does show promise of providing a quality forum for new work in reproductive immunology. □

David Clark is an Associate Professor of Medicine at McMaster University, Hamilton, Ontario.

## Take the strain

Colin J. Barnstable

*Journal of Neuroimmunology*. Editors-in-chief C.S. Raine and P.O. Behan. 6/yr in 2 vols. (Elsevier Biomedical.) Dfl. 450, \$180.

THE new *Journal of Neuroimmunology* (JN) is trying to provide a meeting point for neuropathologists and those who use immunological methods as a tool to study the nervous system. Obviously, all knowledge of the nervous system is relevant to a neuroscientist; but how much does someone working on cell-type specific markers of mouse cerebellar cells *in vitro* (Vol. 1, No.4) have in common with someone working on the distribution of factor B(Bf) allotypes in multiple sclerosis (Vol. 1, No.1)?

Separately, the two target groups of this journal are already well served. There are many neuropathology journals, such as the *Journal of Neuropathology*, whose interests and contents encompass those of JN. Equally, neuroscientists who use immunological methods have a variety of cell and developmental biology journals, as well as several neuroscience journals such as *Brain Research*, *Journal of Neuroscience* and *Journal of Neurochemistry*.

This diversity of journals is as it should be since it is the problem tackled that is important, not the method used.

Although I do not see any particular need for this newcomer, a number of the papers in the first few issues are solid and competent. However since many of them are by the editors, as is often the case for new journals, it is still difficult to judge what the overall quality will be.

The journal is settling down at about six or seven papers in each of the four issues per year. This works out at \$3 per paper, which is more expensive than many equivalent journals. In compensation the quality of the presentation, notably of the electron micrographs, is very good. The delay between receipt and acceptance has varied between 1 day and 5½ months; that between acceptance and publication is about 6 months, although I detect signs of increasing delay in recent issues.

If there is a group of "neuro-immunologists" just waiting to submit high-quality papers to this journal — and take out subscriptions — its future is assured. If not then I hope it will not linger and further strain our already desperate library finances. □

Colin Barnstable is in the Department of Neurobiology at Harvard Medical School, Boston, Massachusetts.

## Strong nerves

Richard J. Miller

*The Journal of Neuroscience*. Editor-in-chief W.M. Cowan. 12/yr. (Williams & Wilkins.) \$80 US, \$100 elsewhere (personal); \$230 US, \$250 elsewhere (institutional). *Cellular and Molecular Neurobiology*. Editor D.O. Carpenter. 4/yr. (Plenum.) \$25 US, \$30 elsewhere (personal); \$50 US, \$57 elsewhere (institutional).

NOWADAYS, there are a great number of scientists who consider themselves to come under the rubric of neurobiologist. Subjects ranging from behavioural psychology on the one hand to molecular biology, immunology and the analysis of bacterial chemotaxis on the other have been applied to the analysis of the nervous system. Given this diversity, publishers and societies seem to take one of two approaches to the problem of how to present information in this area: to publish a journal which deals with specific aspects of the subject, or one which reflects the breadth of the field.

*The Journal of Neuroscience* (JN) is a publication of the latter type. Although the number of new journals one encounters in the library each year seems staggering, it is fair to say that many of these never amount to much. JN is an exception. It has rapidly become a major publication in neurobiology, one to rival the established *Brain Research* and *Neuroscience*; certainly, it is already required reading for anybody who seriously considers himself to be a neuroscientist.

There are several reasons for the warm welcome accorded to JN. One objection many authors have to submitting work to a new journal is that they do not know how widely it will be read or how high its standards will prove to be. In the case of JN this problem never arose since it is the official journal of the Society for Neuroscience and is distributed to all members of the society. Consequently, there has never been any doubt that the journal would be widely available and read by the relevant group of workers.

In addition, high scientific standards



have been ensured by having an editorial advisory board that reads like a *Who's Who* in neurobiology. This board is split into several sections — Molecular, Behavioural, Cellular and Developmental Neuroscience, and Neural Systems — which illustrate the wide range of the journal (purely clinical work, however, is not accepted). It is quite clear from an examination of the journal over the past year that major workers in each of these fields are using the journal for the publication of their best work.



The journal comes out monthly and usually contains about a dozen articles. Considering the breadth of the field it is covering, this means that the editors can be fairly selective, which should help to maintain high standards. Moreover, each issue of the journal is fairly digestible which is not always the case with vast depositories of information such as *Brain Research*. A second attractive feature is that papers are accepted and published quite rapidly (six to nine months) which is reasonable compared to other journals. JN is also quite affordable, being \$60 in 1982 for non-members of the Society for Neuroscience which is good value.

## Cellular and Molecular Neurobiology

One shortcoming of the journal is in the reproduction of plates. In an area where the publication of micrographs is often necessary, JN can be said to do an adequate job but in this respect it is inferior to the superb production of *Neuroscience*. With this one reservation, JN is highly recommended; I am sure it will flourish.

The aims of *Cellular and Molecular Neurobiology* (CMN) are quite different from those of JN. This journal restricts itself to publishing papers analysing neuronal and brain function at the "cellular and subcellular" levels. There is indeed room for a specialized journal in this area.

CMN has had a fairly modest beginning, restricting itself to four issues per year each of which contains six to eight fairly short papers and a couple of short communications. The journal will also publish solicited and unsolicited review articles. The one example of a review to appear so far on "Aminergic Neurons: State Control and Plasticity in Three Model Systems" was quite worthwhile. An attractive feature of the journal is the extremely fast publication time, full papers taking from four to six months from receipt to publication.

A survey of the five issues available for review indicates that most of the articles deal with neuronal biochemistry, receptorology and neurochemistry with a few papers on electrophysiology. The general scientific quality appears to be reasonable and the level of interest fairly high. Although I do not think that as yet the journal is seen by most researchers as the prime target for their work, it holds promise and might be considered by authors in this subject area. Given the modest price this same group might also wish to subscribe. Those in other areas of neurobiology will probably prefer to wait and see how the journal develops. □

Richard Miller is Professor of Pharmacological and Physiological Sciences and Neurobiology at the University of Chicago.

# Paradigms of the paranormal

David Cohen

*Psychoenergetics: The Journal of Psychophysical Systems*. Editor D.F. Lawden. 4/yr. (Gordon & Breach.) £69, \$125.

MOST scientists seem to assume that telepathy, clairvoyance, astrology, metal-bending by mind and other strange phenomena that go bump in the night are nothing but occult bunk. The psychologist Hans Eysenck, no fan of freak beliefs, has recently argued that to refuse to look at the evidence for paranormal claims is itself irrational — and quite unscientific. So the existence of a journal such as *Psychoenergetics* should, in theory, be justifiable.

The journal has recently been revamped, with a change of title (from *Psychoenergetic Systems*) and of editor. It aims to cover work into "any assembly of elements whose behaviour cannot be explained by appeal to the known physical modes of interactions. . .". If mind does affect matter, this is a journal to report it in.

The range of articles is wide, covering most of parapsychology and some physics. Speculative pieces review the nature of time, the nature of consciousness and the

## Psychoenergetics

The Journal of Psychophysical Systems

nature of the observer. For instance, John Valentine develops an interesting comparison between the behaviour of quantum mechanical electrons in solids and the characteristics of consciousness, although his model of consciousness seems to involve little memory and self-awareness.

Many papers, unfortunately, do not reach such a high standard. A 30-page paper investigates the Zen claim that "big minds" are all connected, and the author, Grinberg-Zylberbaum, offers a startlingly literal interpretation of this Zen metaphor about empathy. If "big minds" are all connected, he claims that there must (i) be brain waves travelling from my brain to yours, assuming we have "big minds", and (ii) if two people with "big minds" are together, their EEG records will be similar. The experiments to test these hypotheses involved training subjects to a high pitch of empathy and measuring their EEGs. The paper reports no control group, shows no awareness of experimenter effects and little sense of a literature which suggests that researchers are apt to read EEG records in ways that fit their hypotheses. Moreover, why should empathy mean identical brain waves?

I am sorry to sound stuffy, but precisely because the area is controversial research needs to be meticulous in its methodology. The contributors to *Psychoenergetics* seem curiously unaware of many interesting methodological issues raised by research into the paranormal.

The Society of Psychical Research publishes a technical learned journal, and it is hard to see how *Psychoenergetics* either improves on that or fills a different niche. Despite this criticism, the field of parascience is lively at present and it would not be unreasonable to recommend taking the journal if it were modestly priced. But at £65 for four issues (in 1982) it is super-naturally expensive. □

David Cohen is Editor of Psychology News.

## Mind matters

Stuart Sutherland

*Cognition and Brain Theory*. Editor M.H. Ringle. 4/yr. (Lawrence Erlbaum.) \$18 US, \$27 elsewhere (personal); \$50 US, \$59 elsewhere (institutional.)

OLD journals never die, nor unfortunately do they fade away. That does not prevent new ones emerging, each with a defensive proclamation declaring that it "meets a need". Among other such splendidly hackneyed phrases in the editorial of the first issue of *Cognition and Brain Theory* are: "There is a growing awareness that effective research . . . requires a cooperative, multidisciplinary perspective", "Intended to provide the flexibility necessary for the publication of speculative multidisciplinary research", "The intellectual climate toward this type of innovative study has grown warmer", and "Help to bridge the gap".

The disciplines with which the journal is concerned are philosophy, psychology, linguistics, artificial intelligence and neuroscience, though no paper on the last subject appeared in the 1981 volume, unless one counts as neuroscience an unfinished computer model for detecting optic flow. Several articles merely regurgitate their authors' previous ideas. Thus Chomsky proclaims once more that there are innate mechanisms underlying language acquisition, and Pribram, innocent of any understanding of what vision or hearing must actually compute, yet again hails the hologram as the answer to how the brain works, and indeed to everything else including medicine, economics and politics.

Most of the articles are sensible if not very exciting. They deal mainly with conversation, planning and speech understanding. Almost none of the papers on artificial intelligence and computer simulation is based on a working program and where there is a program, the reader is told



little of its performance and limitations. It is time such papers were banned.

The editors will have to work hard if the journal is to achieve the range of material to which they aspire, particularly as it is in competition with several well-established journals including *Cognition* and *The Behavioral and Brain Sciences*. One should not judge by the first volume, however, since it usually takes a journal a few years to find its identity. There is certainly scope for a multidisciplinary journal containing articles written in such a style that they are

## COGNITION AND BRAIN THEORY

A JOURNAL OF  
PHILOSOPHY • PSYCHOLOGY • LINGUISTICS • ARTIFICIAL INTELLIGENCE • NEUROSCIENCE

all readily assimilable by members of the different disciplines. *Cognition and Brain Theory* should be in your library, but personal subscribers may want to wait a year or two before investing. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex.

## In need of brief analysis?

Geoffrey Hall

*Behaviour Analysis Letters*. Editors J.E.R. Staddon and C.M. Bradshaw. 6/yr. (Elsevier Biomedical.) Dfl. 205, \$82.

THE titles of journals commonly use a sort of code. "Letters" in *Behaviour Analysis Letters* (BAL) is not hard to crack. It means short papers (the editors stipulate 2,500 words maximum), and a short publication lag (running at about 4 months on average for the papers which appear in the latest issue).

A quick survey of my colleagues reveals, however, that not all psychologists are able to decode the phrase "Behaviour Analysis". It means, in fact, that branch of experimental psychology created by B.F. Skinner — the approach that abstains from traditional psychological theory and statistical methodology and concentrates instead upon the detailed study of individual organisms (usually non-human) responding according to the constraints imposed by various schedules of reinforcement. The scope of BAL therefore exactly matches the area currently covered by the well-established *Journal of the Experimental Analysis of Behaviour* (JEAB).

So do we need this new journal? The editors assert that there has long been a want for a journal devoted to short papers on the experimental analysis of behaviour.



Although JEAB (in common with many psychology journals) now prefers work that comes as an "integrative package", some research, argue the editors of BAL, is better presented as a series of short papers. I remain to be convinced. Surely what counts in a paper is not that it is long or short but that it answers some important question (or, very occasionally provokes a

startling new one). Sometimes a short paper can do this, although I did not find an example in the issues of BAL made available for review. Rather, most of the contributions looked indeed like the first of a series and served to make the point that, in this branch of psychology, a single experiment is usually capable only of creating a problem, not of solving it. □

Geoffrey Hall is Senior Lecturer in Psychology at the University of York.

## Primate range

Alan F. Dixon

*American Journal of Primatology*. Editor J. Erwin. 8/yr. (Alan R. Liss.) \$140 US, \$162 Europe.

THERE are now five journals which deal exclusively with primates: *Folia Primatologica*, *Primates*, *Journal of Medical Primatology*, *International Journal of Primatology* (reviewed in *Nature* 293, 350; 1981) and, most recently, the *American Journal of Primatology*.

The new journal has an attractive format and as well as reports of research work carries book reviews and editorials. Manuscripts are reviewed and published promptly. The journal aims to cater for all aspects of primate biology and, as the editor points out, "the breadth of the contributions is remarkable" — in a single issue they range from a paper on gallstones in an aged rhesus monkey to a review on self-awareness and the emergence of mind.

As this is the official journal of the American Society of Primatologists, it should command a good market in the United States. The editorial board is composed entirely of workers in American universities and research institutes, and the

great majority of articles published so far are by American scientists.

Although a broad and comparative approach to studies of primate biology is useful, several existing journals already adopt this policy and it is doubtful whether another publication is warranted. The claim that "knowledge of primates has doubled in less than five years" is also of dubious validity. A landslide of papers is not necessarily commensurate with a doubling in scientific knowledge.

Many of the best studies on primates are published in specialist journals rather than in the primatological literature. This will probably continue to be the case. However, the *American Journal of Primatology* has produced a useful supplement on reproduction and fertility in primates; perhaps the further use of specialized supplements will improve the quality of output achieved by the journal. □

Alan F. Dixon is a Research Fellow at the Institute of Zoology, Regent's Park, London.

## Open issues

Frank Moriarty

*European Applied Research Reports: Environment and Natural Resources Section*. Irregular. (Harwood.) Dfl. 295, \$108.50 per volume of 500 pages. *Environmental Monitoring and Assessment*. Editors G.B. Wiersma and A.I. Sors. 4/yr. (Reidel.) Dfl.66 (personal), Dfl.180 (institutional).

THE reasons for publishing *European Applied Research Reports* are obscure. Volumes in the *Environment and Natural Resources Section* are to have an irregular number of parts, with 400–500 pages per volume, and will contain refereed original scientific work that has been conducted in collaboration with the Commission of the European Communities, reports and symposia proceedings, on all aspects of environmental and natural resources. No instructions are given for would-be authors, and there is no indication of who edits the reports, beyond the fact that they are published for the Directorate-General for Scientific and Technical Information. The standard of production is poor.

Even in the small number of issues yet published, the range of topics is considerable: from causes and remedies for coastal erosion in Togo and Benin to the consequences of phosphorus precipitation in water-purification plants. A great deal of material is included within individual papers that would usually be deleted by most scientific journals. Much of the work is worthy, but the range is so wide, and the essentials so diluted with detail, that the series is best seen as a monument to the

industry of the European Commission.

In contrast, *Environmental Monitoring and Assessment* (EMA) has both well defined aims and named editors. "Environmental monitoring" can mean many things, but the declared objectives restrict consideration to pollutants, with emphasis on scientific principles in the design of monitoring systems, their imple-

## ENVIRONMENTAL MONITORING AND ASSESSMENT

mentation and the consequent assessment of risk to man and the environment.

It is often argued that biological effects are difficult to detect in the field, and that measurements of rates of pollutant release and of degree of environmental contamination can be more meaningful than biological measurements. There are obvious attractions in such an approach, but given that field exposures usually fluctuate, and that we lack information on exposure-effect relationships for fluctuating exposures, this is a big assumption to make. There is the added difficulty, for species other than our own and cultivated and domesticated species, that we are concerned with the effect on populations, not on individuals. One might hope, then, that this journal would give due prominence to consequences.

However, of 20 papers, only three include a significant discussion of biological effects. Of the rest, several take a rather theoretical approach to problems of monitoring: these often contain sensible suggestions, such as the need for clear objectives, and for close cooperation between all members of a survey team, but such ideas are not new.

In brief, it is difficult to evaluate the ideas or work described in many of the papers because there is no evidence on the effectiveness or otherwise with which biological effects are indicated. One paper exemplifies this preoccupation with methods rather than results: 0.5 ha plots of Montana grassland were fumigated with sulphur dioxide for up to five years, but although detailed studies were made of amounts, distribution and fluctuations of sulphur dioxide within and around the vegetation canopy, no observations appear to have been made on the plants' response, although the introduction does list many biological measures to be made with this exposure system. Without that information, the value of the system cannot be evaluated.

For EMA to become a more useful forum on monitoring, it needs to bring its lagging publication schedule up to date and place a greater emphasis on the biological aspects. Given that, it could become a worthwhile venture. □

Frank Moriarty is a Principal Scientific Officer at Monks Wood Experimental Station, NERC Institute of Terrestrial Ecology, Huntingdon.

# Topical tropical entomology from Africa

R. F. Chapman

*Insect Science and Its Application*. Editor Thomas R. Odhiambo. 4/yr. (Pergamon.) £42.50, \$48.57.

NOT content with founding the International Centre for Insect Physiology and Ecology (ICIPE) in Nairobi, Professor Odhiambo has now started the first international entomological journal to stem from black Africa.

The stated intention of *Insect Science* is to "transcend the traditional boundaries of entomology" by its multi-disciplinary approach. The first issue, devoted to papers given at an ICIPE workshop on the epidemiology of African trypanosomiasis, lives up to these aims. Subsequently the papers have lapsed into a more usual entomological mould, though still covering the field from ultrastructure and neurophysiology to ecology and control. Most of the papers, which are generally short descriptive communications, could find a home in various more specialized journals, but many general entomologists especially in the tropics will appreciate having a variety of topics collected together as they in *Insect Science*.

A significant contribution of the journal to date is in publishing papers presented at three workshops, two held at ICIPE on trypanosomiasis and sorghum shoot flies, and one at the University of Ibadan on identification services and taxonomic research centres in Africa. These have been sufficiently wide in scope to be of interest to a wider audience than just those concerned specifically with the topics reviewed.

Although the intention is for the journal to cover entomology in the tropics generally — a point which is not clear from the title — the bias has been very strongly African. This is not necessarily bad, for there is plenty of scope for African entomology. More worrying has been a tendency for *Insect Science* to be something of a house journal for ICIPE. This may have been necessary to get the journal off the ground, but clearly cannot go on if it is to achieve international stature and become a "must" for all those interested in African entomology. Professor Odhiambo will no doubt be aware of this problem and it is to be hoped that he has the international support to overcome it. Assuming that he does so, and

can maintain the standard, this journal will be a worthy addition to the entomological literature. □

R.F. Chapman is an Assistant Director at the Centre for Overseas Pest Research, London.

## European niche

A. d'A. Bellairs

*Amphibia-Reptilia*. Managing co-editors H. Hemmer and A. Dubois. 4/yr. (Akademische Verlagsgesellschaft, Wiesbaden, FRG.) DM 108.

THE appearance of a new journal of authoritative cast, a fit counterpart to some of the better North American productions, is an event of some note for European herpetology.

*Amphibia-Reptilia* is the journal of the Societas Europaea Herpetologica, founded in 1979. It is published in West Germany, with co-editors in Germany and France, and contains original papers of moderate length in English, French, German and Spanish, together with shorter

## Amphibia-Reptilia

Publication of the Societas Europaea Herpetologica

articles, news of its parent society, book reviews and obituaries. The main papers contain abstracts in English and often in another language also.

Well produced and well illustrated in an attractive format, the journal is a professional-looking publication. It deals with a wide variety of topics, including systematics (biochemical and otherwise), genetics, evolution, morphology from the ultrastructural to the gross level, physiology, ecology and behaviour. This catholic approach reflects the fact that herpetology is still more of a coherent discipline than the study of most of the other major groups of vertebrates.

This journal seems to occupy a definite niche in the zoological literature of Europe and is much to be welcomed; together with the *British Journal of Herpetology* in its new, improved form, it should stimulate progress in a subject which seems to be under-represented in many British university departments of zoology. □

A.d'A. Bellairs is Emeritus Professor of Vertebrate Morphology in the University of London and Honorary Consulting Herpetologist to the Zoological Society of London.

## INSECT SCIENCE AND ITS APPLICATION

The International Journal of Tropical Insect Science

## World floras

Peter D. Moore

*Nordic Journal of Botany*. Editor-in-chief M. Lange. 6/yr. (Council for Nordic Publications in Botany.) DKr.480 plus postage.

THE *Nordic Journal of Botany* is a Fenoscandian publication, but the botanical papers it contains are by no means so restricted in their geographical interest. There is a strong emphasis on taxonomic subjects, together with structural botany and taxonomic aspects of mycology, lichenology and phycology. Some of the topics covered are general and wide-ranging, such as proposals for a cladistic classification of green plants; others are very specific in their taxonomic and geographical coverage, the records of ascomycete fungi from northern Thailand for instance.

In order to provide an indication of the relative space assigned to the main subject areas, I have taken a sample of 70 recent papers in the journal and analysed their contents. Almost half of these articles are concerned with taxonomic subjects, mainly of a specific character and largely dealing with angiosperms. Mycological

articles comprise about 20 per cent and lichen and algae papers about 10 per cent each. Only 13 per cent of the papers examined were specifically concerned with anatomy or morphology, and despite the editorial intention to include palaeobotanical subjects no article of this type occurred within the sample. Very few papers are directly concerned with techniques and few involve an experimental approach to taxonomic studies.

Some attention is given to floras of certain under-studied parts of the world, not only dealing with angiosperms, but also with lichens and fungi. Indeed the geographical spread of articles is very wide and over 30 per cent could be regarded as of interest to a phytogeographer. Ecologists, however, will find less of interest here; less than a tenth of the papers have a distinctly ecological slant and most of these concern lichens and fungi.

General production standards are high, including the reproduction of photographs, especially photomicrographs. I feel that the journal could come to play a useful part in the literature of taxonomic botany, particularly if it were to place greater emphasis on experimental studies. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London.

## Water works

J.H.S. Blaxter

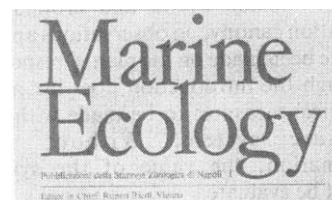
*Aquatic Toxicology*. Editors-in-chief D.C. Malins and A. Jensen. 8/yr in 2 vols. (Elsevier Biomedical.) Dfl.440, \$176. *Marine Ecology*. Editor-in-chief R. Riedl. 4/yr. (Paul Parey, Munich.) DM 380, \$159. *Biological Oceanography*. Editor-in-chief R.L. Haedrich. 4/yr. (Crane, Russak.) \$45 (personal); \$82 (institutional.)

MUCH of the research in marine biology has as its justification the need to preserve the marine environment for its food supplies and for leisure. Strong financial support for studies on pollution by sewage and industrial effluents, eutrophication, insecticides and dumping of nuclear waste has thus rightly been a feature of marine biological work over the past two decades.

Given this it is surprising that a journal such as *Aquatic Toxicology* (AT) was only started in April, 1981. The journal was launched, say the editors, in response to the need for a focus for the publication of "meritorious studies in aquatic toxicology", and they hope to take in all aspects of the mechanisms and assessment of toxicity and the development of techniques in the field and laboratory. The first few issues include many papers on molluscs and fish, especially salmonids and flatfish, with the emphasis being on the effect of hydrocarbons and heavy metals. Certainly such papers were spread over many journals in the past and it seems likely that AT will have a useful centralizing effect in the future.

It will quickly be realized that *Marine Ecology* (ME) is not a new journal but a revamped continuation of *Pubblicazione della Stazione Zoologica di Napoli*, originally founded nearly a century ago. The new name is unfortunate in being similar to another journal (*Marine Ecology, Progress Series* — for review see *Nature* 293, 351; 1981) and to the continuing series of books edited by Otto Kinne; in addition the citation suggested, *PSZNI: Marine Ecology*, is clumsy.

Although the face-lift has been accompanied by a change of editor, the journal



will not change its range of interests very much and is devoted to "promoting scientific knowledge of the sea on a global scale and of the Mediterranean in particular", especially

work on the intimate environment of the sea as exemplified by direct observations on coasts and under sea, long-term investigations and underwater experimentation.

## New outlet for old bones

Barry Cox

*Journal of Vertebrate Paleontology*. Editor J. Zidek. 4/yr. (University of Oklahoma School of Geology.) \$30 (personal); \$60 (institutional).

UNLIKE their invertebrate counterparts, vertebrate palaeontologists are more likely to have had some biological training, and to deal with biological problems, and are less likely to be concerned with stratigraphy. Until now, they have had to publish in general palaeontological journals (*Palaeontology*, for example), in journals concerned with particular geographical areas (*Vertebrata Palasiatica*) or not primarily in the English language (*Paléovertebrata*), or in the small-format journal *Paleobiology* which covers only biological matters.

The *Journal of Vertebrate Paleontology* (JVP) does therefore provide a new opportunity, for it is an English-language, large-format (7"×9" print area) journal that accepts papers on any aspect of the palaeontology of chordates. The typeface is clear and the overall standard of production is high. Text figures, half-tones and plates are printed running along with the two-column text, occupying all or part

of a page. (Greater editorial control is needed here, however, for some line-drawings and plates are too faint or with insufficient contrast, and the mixture of white-background and black-background figures in a single plate is unattractive.) Each issue contains about 120 pages.

Analysis of the first four issues shows that 60 per cent of the papers were short (up to 10 pages) but the range is considerable, the longest having 55 pages. The scientific standard runs the gamut usually found in other journals — so JVP isn't merely publishing papers rejected elsewhere. There have been several book reviews, some of which are excellent, but so far there have been no editorials or comments on papers published in previous issues of the journal.

To date authors are overwhelmingly from North America (31 out of 35 papers), but I am sure that the international editorial board will be working hard to get more contributions from other continents. They should succeed: it's an attractive journal and good value. □

Barry Cox is a Professor in the Zoology Department, King's College, University of London.



ME becomes less of a house journal, then, but should still retain the loyalty of its author clientèle of earlier years. The first five issues show a predominance of central and southern European authors with interests in the Mediterranean. The standard of production is good; here and there an author has even been indulged in the luxury of a colour print. Strangely the journal lacks any detailed instructions for authors.

A completely new journal, *Biological Oceanography*, was launched in 1981 with Dr Richard Haedrich from Newfoundland

## AQUATIC TOXICOLOGY

as the sole editor but supported, as with the other two journals under review, by a strong editorial board. There is no introductory editorial in Vol.1 No.1 but a search under "Information for Authors" shows that the journal aims

to advance understanding of the ocean as a biological system and publishes scientific papers and speculative notes from any branch of biology that contributes to this aim.

(It strikes me that the idea of speculative notes may restrict the authority of any refereeing system!)

The first three issues contain papers, mainly by North American authors, on conventional topics such as vertical migration, ichthyoplankton, marine phyto- and zooplankton and the design of samplers. However an intriguing title, "The distribution of linear rows of holes in the sea floor" is to be found in Vol.1 No.2 — tantalizingly, the maker of these holes was not identified.

A couple of general points may be made about these journals. Two of them require the return of proofs within two or three days of receipt. Surely it is unrealistic to expect scientists working on, say, research vessels to maintain such a strict schedule and, anyway, why should there be such an absurd rush after the author has waited several months for his or her paper to reach the proof stage? While none of the three journals levies page charges *Biological Oceanography* Vol.1 No.1 requests page charges "on a voluntary basis in exchange for additional free reprints" — a strange arrangement indeed (which becomes modified along more conventional lines in later issues).

Professor Rupert Reidl, in a long introductory article to Vol.1 of ME, describes four main phases in the history of marine science — the phase of seafarers, of oceanographic expeditions, of marine stations and of field research. We may add to this a fifth phase — the proliferation of published material. □

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# Physics and physicians

K.V. Ettinger

*Nuclear Medicine Communications.* Editors K.E. Britton, P.J. Ell and R.F. Jewkes. 6/yr. (Chapman and Hall.) £35 UK, £39 elsewhere. *Radiation Protection Dosimetry.* Editor-in-chief T.F. Johns. 8/yr in 2 vols. (Nuclear Technology Publishing, PO Box 7, Ashford, Kent, UK.) £60 UK, \$150 elsewhere. *Clinical Physics and Physiological Measurement.* Honorary editors M.M. Black and P.H. Fentem. 4/yr. (Institute of Physics.) £80 UK, \$150 North America.

NUCLEAR medicine is one of the fastest growth areas of modern medicine, this fact being reflected in the multiple metastases of periodicals devoted to the field. The new arrival, *Nuclear Medicine Communications* (NMC), is a rapid-publication journal sponsored by the British Nuclear Medicine Society and having a particularly impressive editorial board.

The content reflects the traditional scope of nuclear medicine — use of radioisotopes

## Clinical Physics and Physiological Measurement

in medical diagnosis and the management of disease and its follow-up; nuclear magnetic resonance imaging, however, which already claims a number of journals of its own, has still to make its impact felt on the pages of NMC. The journal accepts both clinically and experimentally orientated papers, as well as those concerned with the development of new instrumentation.

Editorials are lively and touch not only on problems of nuclear medicine but also on the intense competition in this and related fields, which appears to spill over from individuals to the scientific societies as a whole. The papers are short and most of the material is likely to appear later in an expanded (and often amended) form in another, non-rapid publication. Apart from the unsightly camera-ready format the standard of production is high. The journal is attracting moderately important contributions, and with its reasonable price it is a worthwhile publication vehicle for physicians, physicists, chemists, physiologists and others who are associated with nuclear medicine.

As journal titles get more specialized, the scope of the scientific field covered is accordingly narrowed. Furthermore, it is easier to search for one's favourite topics in a narrow-range periodical, particularly if computerized abstracting services are not at hand. Such a journal is *Radiation Protection Dosimetry* (RPD), which has reached a high level of scientific competence surprisingly quickly despite stiff competition from *Health Physics, Journal*

of *Applied Radiation and Isotopes*, *Radiation Physics* and several others.

RPD is truly international in scope. Generally, it is addressed to the research community in the field of low-level radiation dosimetry and radiation protection, but some of the material published may be of interest to those involved in the less-loved regulatory side of radiation protection.

In addition to original scientific contributions covering the physical principles, instrumentation and practical implementation of low-level radiation dosimetry, there are well-written reviews of broad interest. Editorials are succinct and deal with scientific problems, not a common feature in specialized scientific periodicals. The subscription rate is high, but not exceptionally so.

*Clinical Physics and Physiological Measurement* (CPPM), the new sister journal to *Physics in Medicine and Biology*, is an official organ of the Hospital Physicists Association (UK), the German Society for Medical Physics and the European Federation of Organizations for Medical Physics. The aim of the editors is to present the latest work in aspects of medical physics, clinical engineering and physiological measurement. In practice the journal's coverage represents a continuation of the spectrum of its companion publication extended to the clinical and physiological side. The European contribution to the journal is hard to spot, but the editors do seem to be succeeding in attracting papers on the intended range of topics. The new European editor, C. Franconi, Rome, himself a rising star of continental medical physics, will undoubtedly improve the European coverage.

In such a broad and varied field it is difficult to judge the scientific quality of the articles, but from those published to date it appears that it is about the average. Good, readable reviews of books, lists of forthcoming events and announcements of professional societies also appear, and the standard of typography and binding is high.

For a long time there was a need for a publication to take the more clinically orientated papers originating from medical physicists, physiologists and biomedical engineers. CPPM is succeeding in this role and is virtually a must for every medical physics library. The price, however, is very steep. A question arises: if this journal and *Physics in Medicine and Biology* were merged into one, would it not be of benefit to both readers and the money conscious librarian? □

K.V. Ettinger is Reader in Bio-Medical Physics and Bio-Engineering at the University of Aberdeen.

# Full of Eastern promise

John Barrow

*Journal of Astrophysics and Astronomy.* General editor S. Ramaseshan. 4/yr. (Indian Academy of Sciences.) Rs 36 (personal); Rs 75 (institutional). *Chinese Astronomy and Astrophysics.* Chief translation editor T. Kiang. 4/yr. (Pergamon.) £100, \$114.28.

I SUPPOSE there are two ways to justify the appearance of a new journal, one geographical, one intellectual. Either you cater for existing disciplines in an area of the world with few indigenous publishing sites or you respond internationally to the emergence of a new discipline or coalition of disciplines. The Indian *Journal of Astrophysics and Astronomy* (JAA) and *Chinese Astronomy and Astrophysics* (CAA) are both reflections of the first provocation.

Each issue of JAA contains about eight research articles with no editorials, or book reviews. The number of non-Indian

## CHINESE ASTRONOMY AND ASTROPHYSICS

(Formerly Chinese Astronomy)

contributors is as yet small and the standard of published papers sound if unspectacular. The printing quality is high but the paper poor and liable to discolour with age. The average time between submission and publication of articles is quite short, about three or four months on average. The success of the journal may well depend on whether foreigners or expatriate Indians submit good articles to it. I suspect they will not. At present this journal is a luxury for science libraries but it is extremely inexpensive and for that reason worth serious consideration.

CAA (formerly *Chinese Astronomy*) is a translation journal covering two Chinese-language journals, *Acta Astronomica Sinica* and *Acta Astrophysica Sinica*. It reflects the rapidly changing nature of Chinese society where perhaps, as in other totalitarian states, no one of intelligence would risk the attempt to do real history, philosophy, sociology or psychiatry, and so the physical and mathematical sciences are of high quality.

The latest issues of the journal reflect keen interest in new ideas in cosmology — massive neutrinos, phase transitions and elementary particles. The 1981 issues include a very interesting overview and bibliography of all the modern Chinese articles in astrophysics. The most impressive articles appearing regularly in the journal are those on the history of astronomy — any scholars with interests in the history of astronomy will find remarkable and otherwise inaccessible archival material published here. All

astronomy and history of science libraries should consider subscribing, especially since the subscription is moderate and over eighty articles appear in an annual volume.

There are just two unfortunate aspects of the journal, consequences of lax editing and proof-reading. First, the article by Ruffini and Gao in Vol. 5, No.1, is a word for word copy of an article by the same authors published earlier in *Physics Letters B*. If the editors and authors consistently ignore copyright they may have problems. Second, the number of misprints among personal names is so high that the uninformed will have unfortunate experiences with the references (also, annoyingly, Soviet references remain in Cyrillic). This aspect of the translation needs to be dramatically improved.

Meanwhile, the references in recent issues double as an entertaining parlour game, "Who's Who in Astrophysics". See if you can identify all the following well-known physicists and astronomers: Carron, McClelland, Lyubimiro, Norikov, Sovel, Saudage (or Sandge), Bawm, Schraman, Radir, Neugebanar, Ress, Morgon, Goldeich, Isreal, Laing, Alven, Berbidge, Saslow, King-Hell, Schener, Clmemnce and Bodenemer. □

John Barrow is a Lecturer at the Astronomy Centre, University of Sussex.

## Physics from Chinese fields

George L. Trigg

*Chinese Physics.* Managing editor A.W.K. Metzner. 4/yr. (American Institute of Physics.) \$370 US, \$377 elsewhere.

MANY of us now active in physics and astronomy in the Western world grew up, as it were, with no thought that significant work in our field might be going on in China. If we did consider the possibility, we probably assumed that whatever research there was would be duly reported in the "standard" journals — *Physical Review*, *Proceedings of the Physical Society*, *Zeitschrift für Physik* or others of that category. Up until relatively few years ago, we would not have been far wrong.

Within roughly the past decade, the picture has changed. Western observers returning from visits to China consistently report significant amounts of physics research, of respectable quality, being

done there. Moreover, especially given the political atmosphere that has existed in China since the fall of the Kuomintang, it is only natural that the Chinese should have inaugurated their own journals. Thus there has grown up a body of literature that is almost completely inaccessible to Western scientists.

*Chinese Physics* represents an effort to remedy the situation. Its purpose is "to make the best current Chinese research in physics and astronomy accessible in a



timely and economical fashion to scientists everywhere who do not read Chinese", by the publication of English translations of selected articles from Chinese journals. The selection is in the hands of an editorial board, each member of which scans one or more of the journals covered (originally nine, extended to thirteen as of Vol.2, No.3). The plan is that eventually the selection will take place in manuscript stage, with Chinese and English publication nearly simultaneous. Until that scheme can be fully implemented, the journal has devoted itself to coverage — still selective — of back issues of the Chinese journals.

I am not in a position to judge the quality of the articles presented. I know the reputations of several members of the editorial board, however, and on that basis I am reasonably confident that it will be comparable to that of "standard" journals. The subject coverage of course reflects the emphasis of Chinese physics and astronomy, which is roughly what one might guess: a modest amount of atomic, molecular and nuclear physics, some theoretical astronomy and astrophysics, some particle theory, some plasma work, and a great deal of condensed-matter physics and optics, with considerable emphasis on application.

There are some deficiencies on the production side of the journal, most of which will be noticed only by the trained eye. One, however, is serious: the reproduction of halftone illustrations is generally poor, sometimes to the point of indecipherability. Another noticeable point is that the page dimensions, at least with the margin allowance used, are fully satisfactory for neither one- nor two-column format. Finally, there are still some traces, occasionally strong, of orientalism in the language, which could be eliminated by a stronger editorial hand.

At the current subscription price, the goal of providing economical access to Chinese research is far from realization, at least on an individual basis. Any serious research institution, however, should have the journal on hand.

George L. Trigg is editor of *Physical Review Letters* for The American Physical Society, Ridge, New York.



# Weather report: outlook fair

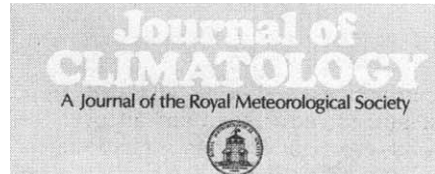
T.M.L. Wigley

*Journal of Climatology*. Editor S. Gregory. 4/yr. (Wiley.) £48.50 UK, \$97.50 elsewhere.

ONLY a few decades ago climatology was seen as a purely descriptive part of meteorology, at best a rather poor second cousin to a true scientific subject. Today few would argue that climatology has emerged as a separate (and expanding) discipline, one having wide ramifications in other fields such as meteorology, hydrology, agriculture, geology, oceanography and astronomy. It is partly because of this breadth of contact that there are few journals which are devoted exclusively to the subject.

In recent years many interesting studies which impinge on climatology have been published in specialist journals in other disciplines, largely because they were directed to those particular specialist audiences. A broad-spectrum climatologist may say, "Ah ha, another paper in my field. No doubt, therefore, of the need for a new journal to gather these contributions together". This is, however, a specious argument, for many such papers merely use the language and, perhaps, some of the techniques or results of climatology.

The *Journal of Climatology*, a journal of the Royal Meteorological Society, does not



cast its net so far, although it has a wide mandate: global and regional studies of climate, local and micro-climatological investigations, changes of climate (past, present and future), and the application of climatological knowledge to a wide range of human activities. The need for such a journal is beyond dispute.

The journal is meant to be international and comprehensive. In the first of these aims it succeeds exceptionally well with an impressive geographical spread of authors in all issues to date and, presumably, a correspondingly international readership. Comprehensive it is not. The majority of contributions in Vol. 1 are synoptic, descriptive studies which largely fall into the old school of climatology. These competent, albeit pedestrian papers certainly warrant publication somewhere, but it is to be hoped that in future issues of the journal the emphasis will shift to a broader front — indeed there is evidence that this is happening. In spite of this criticism of content, the quality of the papers has been uniformly high. This is the main criterion which determines the future

success or failure of a journal and on these grounds success is guaranteed.

The only rival journal is Reidel's *Climatic Change* (for review see *Nature* 293, 362; 1981). While there is some overlap, *Climatic Change* is more multi-

disciplinary and the two journals neatly complement each other. The publishers of the newcomer can learn from *Climatic Change* in one respect, however: the latter offers a low, personal subscription rate to all, whilst the *Journal of Climatology* so favours only members of the Royal Meteorological Society. □

T.M.L. Wigley is Director of the Climatic Research Unit at the University of East Anglia.

## The prospect of PCH

G.K. Batchelor

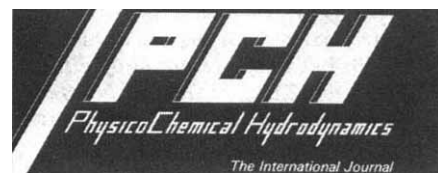
*PhysicoChemical Hydrodynamics*. Editor-in-chief D.B. Spalding. 4/yr. (Pergamon.) £60, \$68.50.

Physico-chemical (or, as the publisher of this new journal chooses to write it, "PhysicoChemical") hydrodynamics may be defined roughly as fluid mechanics with a dash of physical chemistry — phase changes, solute diffusion, interface effects, intermolecular forces, electrokinetic phenomena etc. The term stems from the title of a pioneering book by B.G. Levich (who is chairman of the editorial advisory board of the journal) first published in the USSR in 1952 and republished in English in 1962. This book recorded Levich's own many contributions to the subject, and, more importantly, it made us aware of a new and interesting group of phenomena and processes. The problems in question are essentially fluid dynamical in nature, but the physical chemistry involved puts them outside the competence of many fluid dynamicists.

This is not a coherent field but, as with any interdisciplinary subject, it has a small group of followers identified by their interest and expertise in two different areas. Is this an adequate basis for the launching of a new journal? A vain question, the answer to which would have no bearing on whether *PhysicoChemical*

*Hydrodynamics* will continue to exist or on whether yet more journals will be established.

The layout of editorial material is messy, with five pages at the front and two more at the back in addition to the information



carried on the front cover. There are earnest proclamations ("We have no desire to compete with existing journals, but cater for the narrower specialist" — narrower?); an abundance of good resolutions ("Only SI units will be allowed" — although centimetres, fortunately, and inches, unfortunately, have already made their appearance); and rousing talk about the subject "PCH". A large number of specialist "topic editors" are named, and papers may be submitted directly to them.

No dates of receipt of papers were printed in Vols 1 and 2, but they are appearing in Vol. 3. There are four nominal parts to each (yearly) volume, all dated only with the year; sometimes two parts are merged. The subscription price is about \$1 for three pages — not cheap. The mix of contributions so far is roughly as expected from the title, although there are also some papers on straight H, without the PC, for example on turbulent flow. The editors organize a conference on physico-chemical hydrodynamics every two years, and the papers presented at these conferences are filling up some of the issues.

One can have little idea of the role a new journal will play and the impact it may have, but the decent thing to do is to express a welcome and hope for the best. For *PhysicoChemical Hydrodynamics* it can be said that the prospects are reasonably favourable. □

G.K. Batchelor is Professor of Applied Mathematics at the University of Cambridge.

### Autumn Books

The next review supplement to be published in *Nature* is Autumn Books.

Among the reviewers will be J.W. Cornforth, Stuart Sutherland, Martin Gardner, Michael Ruse, Eric Ashby, Stephen Hawking and Richard Lewontin.

Books to be reviewed include A.G. Cairns-Smith's *Genetic Takeover*, B. Gal-Or's *Cosmology, Physics and Philosophy*, Abraham Pais's *Subtle is the Lord: The Science and Life of Albert Einstein*, John Maynard Smith's *Evolution and the Theory of Games*, Nancy Stepan's *The Idea of Race in Science* and P.B. Medawar's *Pluto's Republic*.

The supplement will appear in the issue of November 11th.



# Pro and con fusion

V.S. Crocker

*Journal of Fusion Energy*. Editor L.M. Lidsky. 6/yr. (Plenum.) \$30 US, \$35 elsewhere (personal); \$80 US, \$91 elsewhere (institutional). *Nuclear Technology/Fusion*. Editor R.G. Post. 4/yr. (American Nuclear Society/European Nuclear Society.) \$100 US, \$127 elsewhere.

THE current situation in fusion has many similarities with that of fission in the 1950s. The subject is burgeoning — a wide variety of fusion devices are being studied, and an even greater number of blanket designs for breeding tritium, producing fissionable isotopes, and for extracting the resulting heat and producing electricity are under investigation. However, there is one difference. No fusion system has yet fully ignited, and perhaps this accounts for the increasing number of articles and meetings on all aspects of fusion technology.

Until 1981 no one publication adequately catered for papers within this wide field, but two — *Journal of Fusion Energy* (JFE) and *Nuclear Technology/Fusion* (NTF) — appeared early in that year to fill this gap.

The content of the journals is almost identical, though NTF normally contains

more pages of text — and costs more. Both magnetic and inertial confined systems are covered, and the range of topics includes plasma engineering, laser developments, magnet technology, tritium handling, blanket neutronics and engineering, materials problems, safety and so on. In general, every aspect of fusion technology for energy — as opposed to basic research — is catered for in both journals.

NTF is a journal of the American Nuclear Society and the European Nuclear Society, and a daughter publication of their *Nuclear Technology*. Besides technical papers, it contains book reviews, technical notes, correspondence and comments on meetings. Also there is a "gallery" of authors' photographs, with potted histories; a feature of doubtful value. Prospective contributors should note that page charges (\$125 per page) are not demanded, though if a cheque is not forthcoming no reprints are available and some delay in publication might occur. At present contributions appear to be published quickly.

JFE seems to be a little more formal with less emphasis on material which questions present ideas (and no photographs of contributors). It has no page charges, and though papers are apparently published rapidly the appearance of issues over the first year has been erratic. Both journals are almost indispensable for scientists and engineers working on fusion.

The standard of papers in the two journals is varied, but usually high. Many contributions contain a number of acronyms, which are hard to decipher; perhaps a start should be made to index these at the end of papers. At present papers from the United States dominate both NTF and JFE and the editors are undoubtedly making efforts to rectify the situation, though the bulk of this type of work is in any case undertaken in the USA.

With fully fledged systems not envisaged until the 1990s and demonstration devices even later, it will be interesting to see if the journals' present momentum can be maintained or if it will fade from exhaustion, as might fusion power reactors! □

V.S. Crocker is Head of the Materials Physics Division at the Atomic Energy Research Establishment, Harwell.

## Sieving on a molecular scale

Rodney P. Townsend

*Zeolites: The International Journal of Molecular Sieves*. Assistant editor Gill Dawson. 4/yr. (Butterworths.) £80 UK, \$208 US.

TO LAUNCH a journal on any subject in the current economic climate is a risky venture; to launch one which deals nearly exclusively with the properties and applications of just one group of porous crystals called zeolites might seem plain foolhardy.

There are exceptions to every rule, however, and *Zeolites* should be one. The growing industrial importance of zeolites in many diverse fields, such as catalytic cracking, gas separation, detergency and pollution control, has resulted in the formation of a large international community of research workers, both academic and industrial. *Zeolites* should therefore provide a natural vehicle for publications on this subject, although many of the more fundamental papers are likely to continue to find their way into well-established journals in physical and inorganic chemistry.

At the time of writing, eight short papers, forty-six full papers and one review had been published since the journal made its debut in April 1981. A good sign is that

contributions are being attracted from established research groups all over the world, especially Belgium, France, Japan and the USA. Papers from most other countries in Western and Eastern Europe have also appeared. In addition to details of research work, each issue contains a very useful Patent Report; the last of these summarized nearly 60 recent patents on zeolites, especially in the areas of catalysis, separation and detergency. Another regular feature is the calendar, which covers forthcoming conferences in related fields.

Presentation is good, with publication times of about six months (which is reasonable, bearing in mind the journal is issued quarterly), although surely it would be possible to publish short papers containing especially interesting new material in the issue immediately following submission.

*Zeolites* will be near-essential to workers in the field and to many industrial departmental libraries. It is likely, however, to be too specialized for subscriptions to be forthcoming from many university libraries. □

Rodney P. Townsend is a Lecturer in Physical Chemistry at the City University, London.



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# Reflections of a Phoenix

P.W. Atkins

*International Reviews in Physical Chemistry*. Editors A.D. Buckingham *et al.* 3/yr. (Butterworths.) £12.50 UK, \$31.25 North America (personal); £45 UK, \$112.50 North America (institutional).

*International Reviews in Physical Chemistry* is the Phoenix which has arisen from the ashes of the *MTP International Review of Science: Physical Chemistry* (a series which expired after its second coming in 1975-76). The initial aim of the new series was to be the same as review journals everywhere: to cater for more or less everyone, from the involved research worker to the passing generalist. As such it was in direct competition with several other publications with readily confusable titles, like the *Annual Review of Physical Chemistry* and *Annual Reports (General and Physical Chemistry)*.

The articles that have appeared so far have ranged widely in length and degree of specialization, although the recent trend, as judged from the small sample yet published, has shifted in favour of length and specialization, and consequently has rendered the reviews only marginally appropriate to the general reader. For the specialist, however, I suspect that the shift is valuable. Instead of coming down firmly between the two stools of general and special readerships, which other publications serve perfectly adequately, the editors are beginning to offer us a journal capable of lengthy reflection on a sequence of specialized fields such as the study of transient species with vacuum ultraviolet photoelectron spectroscopy and rotational fine structure in dynamic photophysical processes. For that there has been a need, and if this journal retains its present format, and is not scuttled by commercial unviability, then it could turn into a substantial and useful contributor to the research scene.

Apart from the review papers the journal offers book reviews, but only infrequently: this service should be extended. As to format and appearance, a half-blind careless reader might think he had picked up a copy of that earlier Phoenix, the *Transactions of the Faraday Society*.

The editors appear to be successful in attracting authoritative contributors: the

publication times are indefinite but appear tolerable. So, in summary: not bad; settling down; useful to specialists; authoritative; moderately expensive; worth watching. □

P. W. Atkins is a Fellow of Lincoln College and Lecturer in Physical Chemistry at the University of Oxford.

## By numbers

W. Graham Richards

*Theochem*. Editors I.G. Csizmadia *et al.* 20/yr in 5 vols. (Elsevier Scientific.) Dfl.950. *Journal of Computational Chemistry*. Editors N.L. Allinger and P. von R. Schleyer. 4/yr. (Wiley.) \$85 US, \$101 elsewhere.

OVER the dead bodies of some traditional chemists, particularly those who devise undergraduate practical courses, a new subdiscipline is growing. This is computational chemistry. It even has its own division within the American Chemical Society. The subject is distinct from theoretical chemistry, the end products being neither theories nor equations but numbers.

The computer is used as a tool rather like a spectrometer. Some applications are computer simulations; others are calculations of molecular energies either from purely empirical potentials or from quantum mechanical calculations, be they semi-empirical or of the *ab initio* variety. Relatively few authors apply the computed molecular wavefunctions to calculate molecular properties other than energies.

At the time of its appearance I greeted *Theochem* (a new journal created by subdividing the *Journal of Molecular Structure*) with some suspicion and declined to be a member of the editorial board on grounds of lack of time. Despite this I have since used the journal both as author and as reader. Clearly the journal has overcome the prejudice of researchers and is now firmly established as a main-line vehicle for publication of calculations of molecular structure.

The range of the *Journal of Computational Chemistry* (JCC) is wider. It encompasses not only molecular structure calculations but also statistical mechanics, Monte Carlo calculations and computer-assisted organic synthesis. Since its inception in 1980, JCC has established itself as the market leader in this broad area of computational chemistry and has become an essential journal in some in-

dustrial contexts as well as in academe.

Both new journals are well produced and seem to have grown beyond the fledgling stage, if only on the evidence that the delay between receipt of a paper and its appearance in print is about a year; this is typical of an established organ. There is no doubt that both will survive and grow fatter as more and more work is produced in the area in which they serve.

That more work will appear is also beyond doubt. Computational chemistry is inextricably linked to development in computer hardware where no obvious threshold in advancement is in sight. Cheaper and more powerful mini-computers, more capable micros with longer word lengths, and the new breed of supercomputers with vector and array processors will all combine to generate more papers for both publications. Their market and readership will indubitably grow, and the editors will be able to maintain high standards.

In one important respect, however, both of these journals and indeed the publishing world in general are not geared to satisfy the needs of the computational chemist. Alongside the advances in computational power has grown a more important hardware availability, that of computer graphics. With the use of colour, the computational chemist can make his work assimilable to the experimentalist and even to boards of directors in scientifically-based companies. Ideally, any journal serving computational chemists should be able to publish graphical output in full colour. Cost is the limitation. To my knowledge a body of fascinating work is being presented at conferences but is never seen by readers of the scientific literature.

The first journal which can overcome this problem will be very attractive to computational chemists. In the meantime *Theochem* and JCC will satisfy most other demands. □

Graham Richards is a Lecturer in Physical Chemistry at the University of Oxford.

## Catalytic work

David J.H. Smith

*Applied Catalysis*. Editor-in-chief B. Delmon. 12/yr in 4 vols. (Elsevier Scientific.) Dfl.820.

THERE has been a rapid growth of activity in catalysis, fuelled by industry's need to conserve energy and husband resources, and it was inevitable that a new journal would be launched to cope with the increase in available material. I'm not sure I know what "applied catalysis" is, but the intended scope of this recent arrival on the

library shelves is wide, encompassing all aspects of catalysis with the emphasis on phenomena connected with industrial catalysts and processes.

*Applied Catalysis* accepts full papers, short communications and even correspondence. In fact, though, all material published so far appears to be in the first category, and almost all of it is devoted to some aspect of heterogeneous catalysis. The scientific standard is high, and the content and style of the contributions is very similar to those that appear in the *Journal of Catalysis*.

I would guess that *Applied Catalysis* was conceived out of the frustration that many authors have felt with the older journal over its very long publication times. So a major aim of the editors is to offer rapid publication which it is hoped will be achieved by using camera-ready copy. This has been partially successful with papers appearing in print about six months after submission. However, as is the case with other journals devoted to catalysis, the acceptance time seems unnecessarily long, being generally at least two months.

An interesting feature is a "News Brief" section collated by J.R.H. Ross from a team of 25 international correspondents

who sometimes seem uncertain whether they are writing for *Transactions of the Faraday Society* or for *Chemical and Engineering News*. Nevertheless it manages to be entertaining and educational, containing tidbits of information on such things as industrial catalyst supports, grants, forthcoming events, excellent book reviews, and even a description of a device for heating shaving foam as it leaves the dispenser.

*Applied Catalysis* already has a secure readership and has developed into a serious competitor for the *Journal of Catalysis*. Its success can be measured by the fact that although it was originally published as one volume of six issues costing \$88.75, in 1982 the intention is to publish three volumes of four issues costing \$200.75. This is at once a sign of good health and an omen for librarians; be warned, if this trend continues it could rival its sister publication the *Journal of Organometallic Chemistry*, now published at the rate of one a week and costing more than \$1,000 per annum. □

David J.H. Smith, presently on secondment to the British Petroleum Research Centre, Sunbury, Middlesex, is a Lecturer in Chemistry at Leicester University.

## Mixed STS

Russell Moseley

*Bulletin of Science, Technology and Society*. Editor-in-chief Rustum Roy. 6/yr. (Pergamon.) £42.50, \$48.50.

FOR some years now the area of study known as science, technology and society has been catered for by such journals as *Social Studies of Science*, *Research Policy*, *Radical Science Journal* and *Science and Public Policy*. This is not to mention the orthodox history and philosophy of science publications, and such journals as *Minerva*, *Studies in Science Education* and the like. Why, then, is there felt to be a need for yet another publication in this field?

Most obligingly the editors of the *Bulletin of Science, Technology and Society* (BSTS) list a number of reasons: the journal provides a place where shorter papers on science studies can be published rapidly; it aims to become the principal house journal of the emerging science studies community; and it publishes a wide range of educational modules designed specifically for teaching purposes. In this last respect BSTS is, as the editors point out, something of an innovation being a hybrid between a textbook and a journal. It could of course equally well be seen as neither a textbook nor a journal.

The success of BSTS in meeting its stated aims is, on the basis of Vol. 1, patchy at best. It has certainly succeeded in publishing shorter papers (only two of which are by authors who are not members of the editorial board) on a wide range of topics, although these comprise only 20 per cent of the whole of Vol. 1. Even less space is devoted to "News and Reviews" (less than 4 per cent of the first volume), and the editors' hope that "lively debate among authors and readers" will occur is, on the available evidence, unlikely to be realized: the first six issues contain not a single letter from a reader.

On the other hand the ample provision of educational material has certainly been accomplished, and has possibly been carried to excess: no less than 77 per cent of Vol. 1 is composed of reprinted course units which are intended to be used for educational purposes. Much of this material has been available for some time and it is debateable whether subscribing to BSTS is the most cost-effective way of building up a collection.

Does the journal fill a gap? To an extent it does by operating at a more popular, accessible level than some of the competing publications. But its hybrid form means it is in danger of slipping through the gap and disappearing. I hope it develops a more tenacious hold in subsequent volumes. □

Russell Moseley is Assistant Registrar for Science with the Council for National Academic Awards, London.

## On the right track?

David Briggs

*TrAC (Trends in Analytical Chemistry)*. Editor P.T. Shepherd. 12/yr. (Elsevier Scientific.) £22 UK, \$40 North America, Dfl.105 Europe (personal); Dfl.320 UK, North America and Europe, Dfl.334 elsewhere (institutional + compendium).

THE stated aim of *Trends in Analytical Chemistry* is

to promote an awareness of the latest trends and developments in analytical chemistry, not only amongst analytical chemists themselves, but amongst all those who use or must be familiar with analytical methods.

The style is that of a magazine with short articles of the news/comment/interest/review type, together with about five (invited) review papers, each on average no more than five pages in length. Each issue is therefore rather thin. The presentation is clear but there is no standardization of the style of figures or illustrations. Cartoons are occasionally included, even in the reviews, presumably to lighten the reading matter. This feature, however, is apparently to be dropped.

It is difficult to believe that the gap which the publishers of *TrAC* are hoping to fill — that for a general awareness bulletin — really exists. Analytical chemistry is a wide field and has several well-developed branches such as spectroscopy, chromatography and chemical assay. Most

professional analytical chemists are specialists either in one of these branches or in a particular field of application, both types already being well served by primary and review literature sources. Many, if not most, of the reviews in *TrAC* merely duplicate this material.

# TRAC

On the other hand, the journal *Analytical Chemistry* still manages to straddle the field and does an admirable job of providing regular "trend" reviews. There are also a number of publications which provide general awareness information for the non-specialist, including the plethora of free bulletins produced by the instrument manufacturers.

In principle there might be a place for *TrAC* in the mail-boxes of personal subscribers, but when compared with the alternative sources it does not seem to offer value for money. On the basis of existing evidence, then, it is hard to see that *TrAC* is making any significant addition to the literature currently available. □

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## REVIEW ARTICLE

## Exploration of the primary visual cortex, 1955–78

David H. Hubel\*

*The following article is the lecture delivered by the author in Stockholm on 8 December 1981 when he received the Nobel Prize in Medicine or Physiology which he shared with Roger Sperry and Torsten Wiesel. The article is published here with permission from the Nobel Foundation and will also be included in the complete volume of Les Prix Nobel en 1981 as well as in the series Nobel Lectures (in English) published by Elsevier. The lecture of Dr Wiesel will appear in next week's issue.*

IN the early spring of 1958 I drove over to Baltimore from Washington DC, and in a cafeteria at Johns Hopkins Hospital met Stephen Kuffler and Torsten Wiesel for a discussion that was more momentous for Torsten's and my future than either of us could have possibly imagined.

I had been at Walter Reed Army Institute of Research for 3 years, in the Neuropsychiatry Section headed by David Rioch, working under the supervision of M. G. F. Fuortes. I began at Walter Reed by developing a tungsten microelectrode and a technique for using it to record from cats with permanently implanted electrodes, and I had been comparing the firing of cells in the visual pathways of sleeping and waking animals.

It was time for a change in my research tactics. In sleeping cats, only diffuse light could reach the retina through the closed eyelids. Whether the cat was asleep or awake, diffuse light failed to stimulate the cells in the striate cortex. In waking animals I had succeeded in activating many cells with moving spots on a screen, and had found that some cells were very selective in that they responded to movement when a spot moved in one direction across the screen (for example, from left to right) but not when it moved in the opposite direction<sup>1</sup> (Fig. 1). There were many cells that I could not influence at all. Obviously there was a gold mine in the visual cortex, but methods were needed that would permit recording of single cells for many hours, and with the eyes immobilized, if the mine were ever to begin producing.

I had planned to do a postdoctoral fellowship at Johns Hopkins Medical School with Vernon Mountcastle, but the timing was awkward for him because he was remodelling his laboratories. One day Kuffler called and asked if I would like to work in his laboratory at the Wilmer Institute of Ophthalmology at the Johns Hopkins Hospital with Torsten Wiesel, until the remodelling was completed. That was expected to take about a year. I did not have to be persuaded; some rigorous training in vision was just what I needed, and though Kuffler himself was no longer working in vision the tradition had been maintained in his laboratory. Torsten and I had visited each other's laboratories and it was clear that we had common interests and similar outlooks. Kuffler suggested that I come over to discuss plans, and that was what led to the meeting in the cafeteria.

It was not hard to decide what to do. Kuffler had described two types of retinal ganglion cells which he called 'ON-centre' and 'OFF-centre'. The receptive field of each type was made up of two mutually antagonistic regions, a centre and a surround, one excitatory and the other inhibitory. In 1957, Barlow, FitzHugh and Kuffler had gone on to show that, as a consequence, retinal ganglion cells are less sensitive to diffuse light than to a spot just filling the receptive-field centre<sup>2</sup>. It took me some time to realize what this meant: that the way a cell responds to any visual scene will change very little when, for example, the sun goes behind a cloud and the light reflected

from black and white objects decreases by a large factor. The cell virtually ignores this change, and our subjective assessment of the objects as black or white is likewise practically unaffected. Kuffler's centre-surround receptive fields thus began to explain why the appearance of objects depends so little on the intensity of the light source. Some years later Edwin Land showed that the appearance of a scene is similarly relatively independent of the exact wavelength composition of the light source. The physiological basis of this colour independence has yet to be worked out.

The strategy (to return to our cafeteria) seemed obvious. Torsten and I would simply extend Stephen Kuffler's work to the brain; we would record from geniculate cells and cortical cells, map receptive fields with small spots, and look for any further processing of the visual information.

My reception in Kuffler's office the first day was memorable. I was nervous and out of breath. Steve, at his desk, rotated around in his chair and said "Hi David! Take off your coat, hang up your hat, do up your fly". His manner was informal! But it took me a month, given my Canadian upbringing, to force myself to call him Steve. For the first three months no paycheck arrived, and finally I screwed up the courage to go in and tell him. He laughed and laughed, and then said, "I forgot!"

Torsten and I did not waste much time. Within a week of my coming to Hopkins (to a dark and dingy inner windowless room at the Wilmer Institute basement, deemed ideal for visual studies) we did our first experiment. For the time being we finessed the geniculate (at Walter Reed I had convinced myself that the cells were centre-surround) and began right away with cortex. The going was rough. We had only the equipment for retinal stimulation and recording that had been designed a few years before by Talbot and Kuffler<sup>3</sup>. A piece of apparatus resembling a small cyclotron held the anaesthetized and paralysed cat with its head facing almost directly upward. A modified ophthalmoscope projected a background light and a spot stimulus onto the retina. The experimenter could look in, see

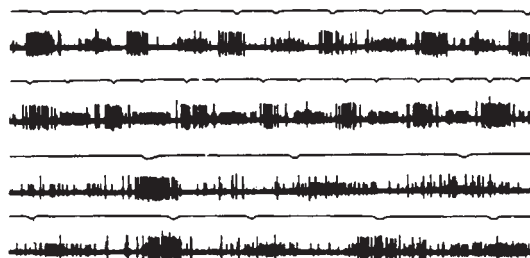
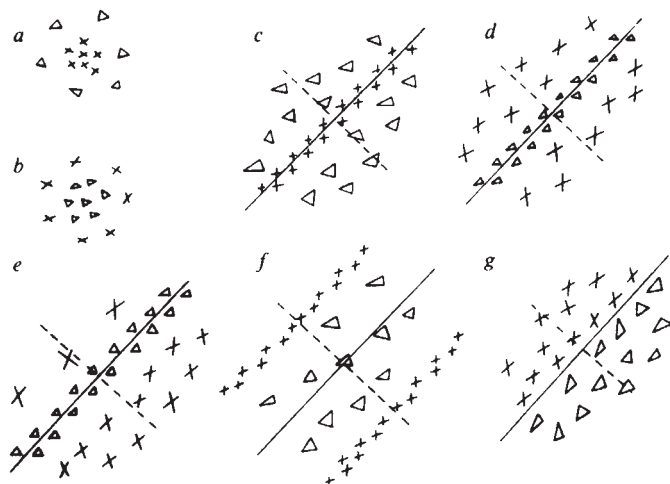


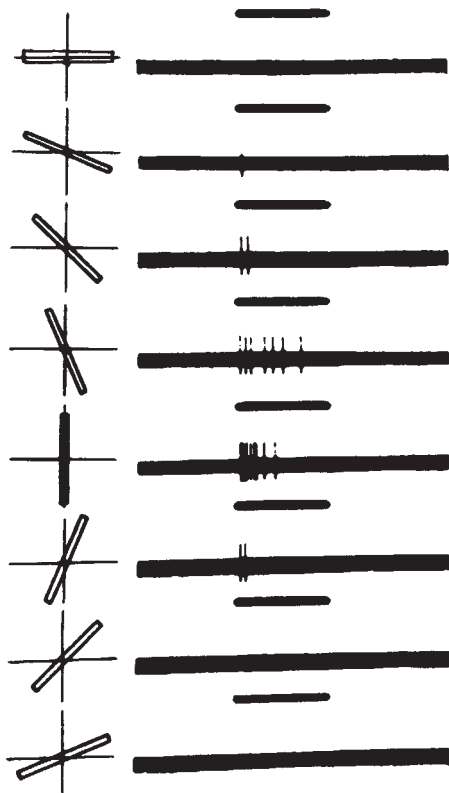
Fig. 1 Continuous recording from the striate cortex of an unrestrained cat. In each dual trace, the lower member shows the microelectrode oscilloscope recording from two cells, one with large impulses, the other with smaller ones. The stimulus was small to-and-fro hand movements in front of the cat. Each movement interrupted a light beam falling on a photoelectric cell, producing the notches in the upper beam. The upper two pairs of records represent fast movements, the lower ones slower movements. Each line represents 4 s<sup>1</sup>.

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**Fig. 2** Common arrangements of lateral geniculate (a, b) and simple cortical (c-g) receptive fields. Symbols: X, areas giving excitatory (ON) responses;  $\Delta$ , areas giving inhibitory (OFF) responses. Receptive-field orientations are shown by continuous lines through field centres; in this figure these are all oblique, but each arrangement occurs in all orientations (Fig. 2 in ref. 10).

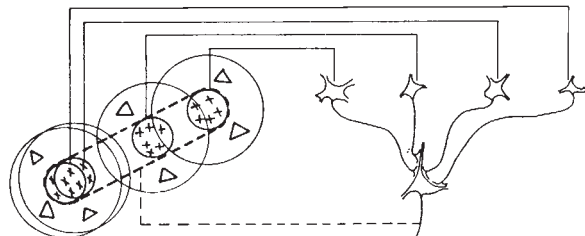
the retina with its optic disk, area centralis, and blood vessels, and observe the background light and the stimulus spots. Small spots of light were produced by sliding  $2 \times 5$  cm metal rectangles containing various sizes of holes into a slot in the apparatus, just as one puts a slide into a slide projector. To obtain a black spot on a light background one used a piece of glass like a microscope slide, onto which a black dot had been glued. All this was ideal for stimulating the retina and recording directly from retinal ganglion cells, since one could see the electrode tip and know where to stimulate, but for cortical recording it was horrible. Finding a receptive field on the retina was difficult, and we could never remember what part of the retina we had stimulated. After a month or so we decided to have the cat



**Fig. 3** Responses to shining a rectangular slit of light  $1^\circ \times 8^\circ$ , so that centre of slit is superimposed on centre of receptive field in various orientations. Receptive field is of type C (see Fig. 2), with the axis vertically oriented (Fig 3 in ref. 12).

face a projection screen, as I had at Walter Reed and as Talbot and Marshall had in 1941<sup>4</sup>. Having no other head holder, we continued for a while to use the ophthalmoscope's head holder, which posed a problem since the cat was facing directly up. To solve this we brought in some bed sheets which we slung between the pipes and cobwebs that graced the ceiling of the Wilmer basement, giving the setup the aura of a circus tent. On the sheets we projected our spots and slits. One day Mountcastle walked in on this scene, and was horror struck at the spectacle. The method was certainly inconvenient since we had to stare at the ceiling for the entire experiment. Then I remembered having seen in Mountcastle's laboratory a Horsley-Clarke head holder that was not only no longer being used but also had the name of the Wilmer Institute engraved on it. It was no other than the instrument that Talbot had designed for visual work when he and Marshall mapped out visual areas I and II in the cat in 1941<sup>4</sup>. For years Vernon had used it in his somatosensory work, but he had recently obtained a fancier one. Torsten and I decided to reclaim the Wilmer instrument, not without some trepidation. To give ourselves confidence we both put on lab coats, for the first and last times in our lives, and looking very professional walked over to Physiology. Though Mountcastle was his usual friendly and generous self, I suspect he was loath to part with this treasure, but the inscription on the stainless steel was not to be denied and we walked off with it triumphantly. It is still in use (now at Harvard; we literally stole it from the Wilmer), and has probably the longest history of uninterrupted service of any Horsley-Clarke in the world.

A short while before this adventure we had gone to a lecture by Vernon (this was a few years after the discovery of cortical columns)<sup>5</sup> in which he had amazed us by reporting on the results



**Fig. 4** Possible scheme for explaining the organization of simple receptive fields. A large number of lateral geniculate cells, of which four are illustrated on the right, have receptive fields with ON-centres arranged along a straight line on the retina. All of these project onto a single cortical cell, and the synapses are supposed to be excitatory. The receptive field of the cortical cell will then have an elongated ON-centre, indicated by the interrupted lines in the receptive-field diagram (left) (Fig. 19 in ref. 10).

of recording from some 900 somatosensory cortical cells, for those days an astronomic number. We knew we could never catch up, so we catapulted ourselves to respectability by calling our first cell No. 3000 and numbering subsequent ones from there. When Vernon visited our circus tent we were in the middle of a three-unit recording, cells number 3007, 3008 and 3009. We made sure that we mentioned their identification numbers. All three cells had the same receptive-field orientation, but neither Vernon nor we realized, then, what that implied.

Our first real discovery came about as a surprise. We had been doing experiments for about a month. We were still using the Talbot-Kuffler ophthalmoscope and were not getting very far; the cells simply would not respond to our spots and annuli. One day we made an especially stable recording. [We had adapted my technique for recording, which used long-term implantations and the Davies closed chamber<sup>6</sup>, to the short-term experiments, and no vibrations short of an earthquake were likely to dislodge things.] The cell in question lasted 9 hours, and by the end we had a very different feeling about what the cortex might be doing. For 3 or 4 hours we got

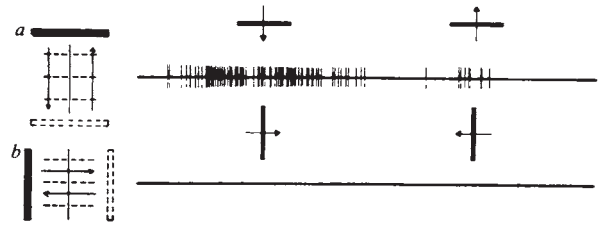


absolutely nowhere. Then gradually we began to elicit some vague and inconsistent responses by stimulating somewhere in the midperiphery of the retina. We were inserting the glass slide with its black spot into the slot of the ophthalmoscope when suddenly over the audiometer the cell went off like a machine gun. After some fussing and fiddling we found out what was happening. The response had nothing to do with the black dot. As the glass slide was inserted its edge was casting onto the retina a faint but sharp shadow, a straight dark line on a light background. That was what the cell wanted, and it wanted it, moreover, in just one narrow range of orientations.

This was unheard of. It is hard, now, to think back and realize just how free we were from any idea of what cortical cells might be doing in an animal's daily life. That the retinas mapped onto the visual cortex in a systematic way was, of course, well known, but it was far from clear what this apparently unimaginative remapping was good for. It seemed inconceivable that the information would enter the cortex and leave it unmodified, especially when Kuffler's work in the retina had made it so clear that interesting transformations took place there between input and output. One heard the word 'analysis' used to describe what the cortex might be doing, but what one was to understand by that vague term was never spelled out. In the somatosensory cortex, the only other cortical area being closely scrutinized, Mountcastle had found that the cells had properties not dramatically different from those of neurones at earlier stages.

Many of the ideas about cortical function then in circulation seem in retrospect almost outrageous. One has only to remember the talk of 'suppressor strips', reverberating circuits, or electrical field effects. This last notion was taken so seriously that no less a figure than our laureate-colleague Roger Sperry had had to put it to rest, in 1955, by dicing up the cortex with mica plates to insulate the subdivisions, and by skewering it with tantalum wire to short out the fields, neither of which procedures seriously impaired cortical function<sup>7,8</sup>. Nevertheless, the idea of ephaptic interactions was slow to die out. There were even doubts as to the existence of topographic representation, which was viewed by some as a kind of artefact. One study, in which a spot of light projected anywhere in the retina evoked potentials all over the visual cortex, was interpreted as a refutation of topographic representation, but the result almost certainly came from working with a dark-adapted cat and a spot so bright that it scattered light all over the retina. It is surprising, in retrospect, that ideas of nonlocalization could survive in the face of the masterly mapping of visual fields onto the cortex in rabbit, cat and monkey done by Talbot and Marshall far back in 1941<sup>4</sup>.

It took us months to convince ourselves that we were not at the mercy of some optical artefact, such as anyone can produce by squinting their eyes and making vertical rays emanate from street lights. We did not want to make fools of ourselves quite



**Fig. 6** Same cell as for Fig. 5, showing response to a moving horizontal bar. *a*, Downward movement was superior to upward; *b*, there was no response to a moving vertical bar (Figs 7 and 8 in ref. 10).

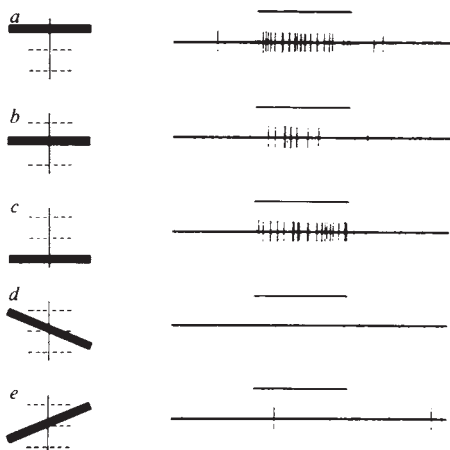
so early in our careers. But recording in sequence in the same penetration several cells, with several different optimal orientations would, I think, have convinced anyone. By January we were ready to take the cells we thought we could understand (we later called them 'simple cells') and write them up. Then, as always, what guided and sustained us was the attitude of Kuffler, who never lectured or preached but simply reacted with buoyant enthusiasm whenever he thought we had found something interesting, and acted vague and noncommittal when he found something dull.

### Hierarchy of visual cells

During the years 1959–62, first at the Wilmer Institute and then at Harvard Medical School, we were mainly concerned with comparing responses of cells in the lateral geniculate body and primary visual cortex of the cat. In the lateral geniculate we quickly confirmed my Walter Reed finding that the receptive fields are like those of retinal ganglion cells in having an antagonistic concentric centre-surround organization. But now we could directly compare the responses of a geniculate cell with those of a fibre from an afferent retinal ganglion cell, and we found that in geniculate cells the power of the receptive-field surround to cancel the input from the centre was increased. This finding was subsequently confirmed and extended in a beautiful set of experiments by Cleland *et al.*<sup>9</sup>, and for many years it remained the only known function of the lateral geniculate body.

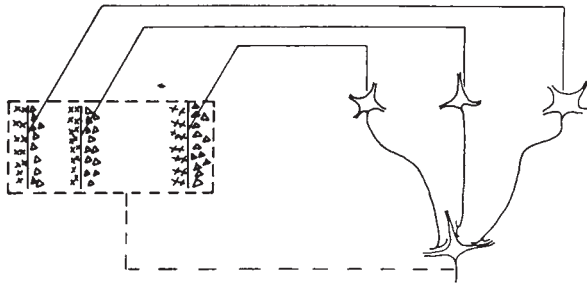
In the cat striate cortex it soon became evident that the cells were more complex than geniculate cells, and came in several degrees of complexity<sup>10</sup>. One set of cells could be described by techniques similar to those used in the retina by Kuffler; we called these 'simple'<sup>11,12</sup>. Their receptive fields, like the fields of retinal ganglion cells and of lateral geniculate cells, were subdivided into antagonistic regions, illumination of any one of which tended to increase or decrease the rate of firing. But simple cells differed from retinal ganglion cells and lateral geniculate cells in the striking departure of their receptive fields from circular symmetry; instead of a single circular boundary between centre and surround, the antagonistic subdivisions were separated by parallel straight lines whose orientation (vertical, horizontal or oblique) soon emerged as a fundamental property (Fig. 2). The optimal stimulus—a slit, dark bar or edge—was easily predictable from the geometry of the receptive field, so that a stationary line stimulus worked optimally when its boundaries coincided with the boundaries of the subdivisions (Fig. 3), and displacing the line to a new position parallel to the old one generally resulted in a sharp decline in the response. Perhaps most remarkable was the precision of the spatial distribution of excitatory and inhibitory effects: not only did diffuse light produce no response (as though the excitatory and inhibitory effects were mutually cancelling with the precision of an acid-base titration), but any line oriented 90° to the optimal was also without effect, regardless of its position along the field, suggesting that the subpopulations of receptors so stimulated also had precisely mutually cancelling effects.

In the cat, simple cells are mostly found in layer 4, which is the site of termination of the bulk of the afferents from the lateral geniculate body. The exact connections that lead to orientation specificity are still unknown, but it is easy to think



**Fig. 5** *a-c*, Complex cell responding best to a black, horizontally oriented rectangle placed anywhere in the receptive field. *d*, *e*, Tilting the stimulus rendered it ineffective.





**Fig. 7** Possible scheme for explaining the organization of complex receptive fields. A number of cells with simple fields, of which three are shown schematically, are imagined to project to a single cortical cell of higher order. Each projecting neurone has a receptive field arranged as shown to the left: an excitatory region to the left and an inhibitory region to the right of a vertical straight-line boundary. The boundaries of the fields are staggered within an area outlined by the interrupted lines. Any vertical-edged stimulus falling across this rectangle, regardless of its position, will excite some simple-field cells, leading to excitation of the higher-order cell (Fig. 20 in ref. 10).

of plausible circuits. For example, the behaviour of one of the commonest kinds of simple cells may be explained by supposing that the cell receives convergent excitatory input from a set of geniculate cells whose ON-centres are distributed in overlapping fashion over a straight line (Fig. 4). In the monkey, the cells of layer 4C (where most geniculate fibres terminate) all seem to be concentric centre-surround, and the simple layers immediately superficial to 4C. No one knows why this extra stage of centre-surround cells is intercalated in the monkey's visual pathway.

The next set of cells we called 'complex' because their properties cannot be derived in a single logical step from those of lateral geniculate cells (or, in the monkey, from the concentric cells of layer 4C). For the complex cell (compared with the simple cell), the position of an optimally oriented line need not be so carefully specified: the line works anywhere in the receptive field, evoking about the same response wherever it is placed (Fig. 5). This can most easily be explained by supposing that the complex cell receives inputs from many simple cells, all of whose receptive fields have the same orientation but differ slightly in position (Fig. 7). Sharpness of tuning for orientation varies from cell to cell, but the optimal orientation of a typical complex cell in layer 2 or 3 in the monkey can be easily determined to the nearest 5–10°, with no more stimulating equipment than a slide projector and a screen.

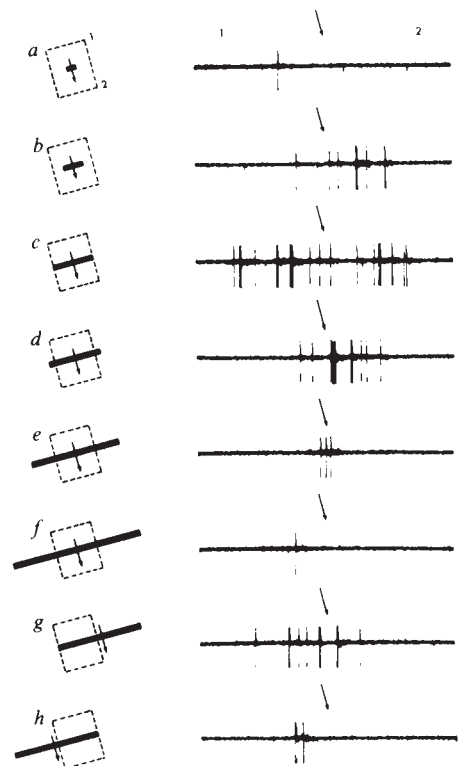
For a complex cell, a properly oriented line produces especially powerful responses when it is swept across the receptive field (Fig. 6). The discharge is generally well sustained as long as the line keeps moving, but falls off quickly if the stimulus is stationary. About half of the complex cells fire much better to one direction of movement than to the opposite direction, a quality called 'directional selectivity', which probably cannot be explained by any simple projection of simple cells onto complex cells, but seems to require inhibitory connections with time delays of the sort proposed for rabbit retinal ganglion cells by Barlow and Levick<sup>13</sup>.

Many cat or monkey cells, perhaps 10 to 20% in area 17, respond best to a line (a slit, an edge, or a dark bar) of limited length; when the line is prolonged in one direction or both, the response falls off. This is called 'end stopping'. In some cells the response to a very long line fails completely (Fig. 8)<sup>14</sup>. We originally called these cells 'hypercomplex' because we looked on them as next in an ordered hierarchical series, after the simple and the complex. We saw hypercomplex cells first in areas 18 and 19 of the cat, and only later in area 17. Dreher subsequently found cells, in all other ways resembling simple cells, that showed a similar fall-off in response as the length of the stimulus exceeded some optimum<sup>15</sup>. It seems awkward to call these cells hypercomplex: they are probably better termed

'simple end-stopped', in contrast to 'complex end-stopped'.

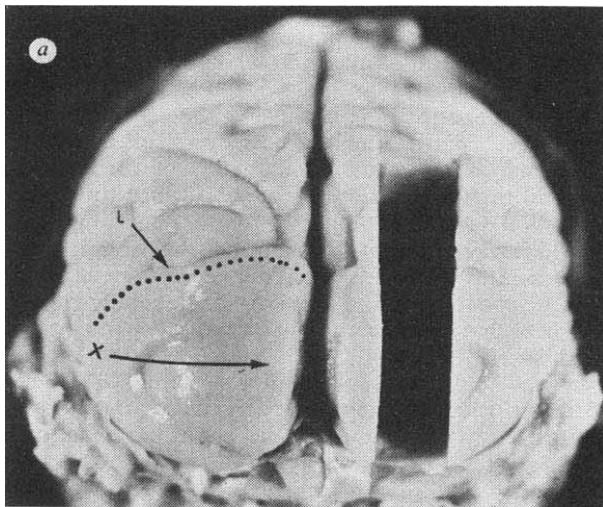
Complex cells come in a wide variety of subtypes. Typical cells of layers 2 and 3 have relatively small receptive fields and low spontaneous activity, and in the monkey may be not only highly orientation-selective but also fussy about wavelength, perhaps responding to red lines but not white. They may or may not be end-stopped. Cells in layers 5 and 6 have larger fields. Those in layer 5 have high spontaneous activity, and many respond just as well to a very short moving line as to a long one. Many cells in layer 6 respond best to very long lines<sup>16</sup>. These differences are doubtless related to the important fact, first shown with physiological techniques by Toyama *et al.*<sup>17</sup> and confirmed and extended by anatomical techniques, that different layers project to different destinations—the upper layers mainly to other cortical regions; layer 5 to the superior colliculus, pons and pulvinar; and layer 6 back to the lateral geniculate body and to the claustrum.

In the past 10 or 15 years the subject of cortical receptive-field types has become rather a jungle, partly because the terms simple and complex are used differently by different people and partly because the categories themselves are not cleanly separated. Our original idea was to emphasize the tendency towards increased complexity as one moves centrally along the visual path and the possibility of accounting for a cell's behaviour in terms of its inputs. The circuit diagrams we proposed were just a few examples from a number of plausible possibilities. Even today the actual circuit by which orientation specificity is derived from centre-surround cells is unknown, and indeed the techniques necessary for solving this may still not be available. One can nevertheless say that cells of different complexities whose receptive fields are in the same part of the visual field and which have the same optimal orientation are likely to be interconnected, whereas cells with different optimal orientations are far less likely to be interconnected. In the monkey, a major difficulty with the hierarchical scheme outlined here is the relative scarcity of simple cells, compared with the huge numbers of cells with concentric fields in layer 4C or with the large number of complex cells above and below layer 4.



**Fig. 8** Hypercomplex cell responding to a black bar oriented from 2:30 to 8:30, moving downward. The optimum response occurred when a stimulus swept over area outlined (c); stimulating more than this region (d–h), or less (a or b), resulted in a weaker response. Sweep duration, 2.5 s (Fig. 19 in ref. 14).





**Fig. 9** *a*, Brain of a macaque, pertused with formalin, viewed from above and behind. The occipital lobe is demarcated in front by the lunate sulcus (L) and consists mainly of the striate cortex, area 17, which occupies most of the smooth surface, extending forward to the dotted line (the 17–18 border). If followed medially, area 17 curves around the medial surface of the brain and continues in a complex buried fold, a part of which lies underneath the convexity and parallel to it. X marks the projection of the fovea; movement in the direction of the arrow corresponds to movement along the horizon; movement along the dotted line, to movement down along the vertical midline of the visual field. The groove in the right hemisphere was made by removing a parasagittal block of tissue to produce the cross-section of *b* (Fig. 6a in ref. 29). *b*, Low power Nissl-stained section from a parasagittal block. It is what would be seen if one could stand in the groove of *a* and look to the left. A, outer convexity; B, the buried fold; arrows indicate the 17–18 borders, the upper right one of which is indicated by the dotted line in *a* (Fig. 6b in ref. 29). *c*, Cross-section through the monkey striate cortex stained with cresyl violet and showing conventional layering designations. W, white matter. Deeper layers (5 and 6) of the buried fold of the cortex are shown in the lower part of the figure (compare *b*) (Fig. 10 in ref. 29).



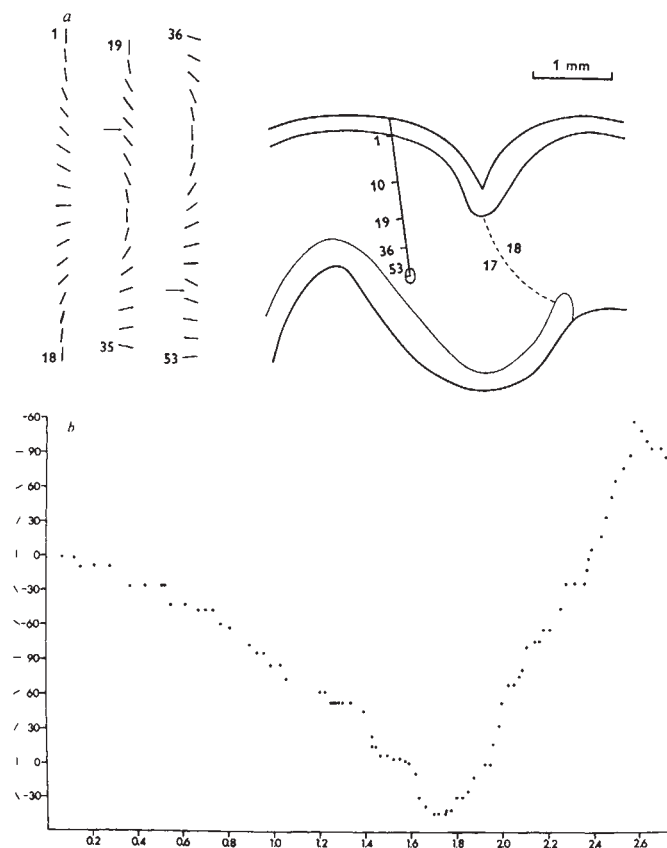
The fact that the simple cells have been found mainly in layer 4B also agrees badly with Jennifer Lund's finding that layer 4C $\beta$  projects not to layer 4B but to layer 3. One has to consider the possibility that in the monkey the simple-cell step may be skipped, perhaps by summing the inputs from cells in layer 4 on dendrites of complex cells. In such a scheme each main dendritic branch of a complex cell would perform the function of a simple cell. All such speculation only emphasizes our ignorance of the exact way in which the properties of complex cells are built up.

Knowing how cortical cells respond to some visual stimuli and ignore others allows us to predict how a cell will react to any given visual scene. Most cortical cells respond poorly to diffuse light, so that when I gaze at a white object, say an egg, on a dark background I know that those cells in my area 17 whose receptive fields fall entirely within the boundaries of the object will be unaffected. Only the fields that are cut by the borders of the egg will be influenced, and then only if the local orientation of a border is about the same as the orientation of the receptive field. Slightly changing the position of the egg without changing its orientation will produce a dramatic change in the population of activated simple cells, but a much smaller change in the activated complex cells.

Orientation-specific simple or complex cells are specific for the direction of a short line segment. The cells are thus best not thought of as line detectors: they are no more line detectors than they are curve detectors. If our perception of a certain line or curve depends on simple or complex cells it presumably depends on a whole set of them, and how the information from such sets of cells is assembled at subsequent stages in the path to build up what we call percepts of lines or curves (if indeed anything like that happens at all) is still a complete mystery.

## Architecture

By the early 1960s, our research had extended into four different but overlapping areas. Closest to conventional neurophysiology was the working out of response properties (receptive fields) of single cells. We became increasingly involved with architecture—the grouping of cells according to function into layers and columns, studied by electrode track reconstructions. This led in turn to experiments in which single-cell recording was combined with experimental anatomy. It began when one day James Sprague called to tell us that his chief histological technician, Jane Chen, was moving to Boston and needed a job: could we take her? Luckily we did (despite our not possessing anatomical union cards), and so acquired an expert in the Nauta method of making lesions in nervous tissue and selectively staining the degenerating axons. It seemed a terrible waste not to use this method and we soon got the idea of working out detailed pathways by making microelectrode lesions that were far smaller than conventional lesions and could be precisely placed by recording with the same electrodes. It became possible to make lesions in single layers of the lateral geniculate body, with results to be discussed shortly. Finally, another phase of our work involved studies of newborn animals' postnatal development and the effects of distorting normal sensory experience in young animals. This began in 1962 and grew steadily. Torsten Wiesel will discuss these experiments in the next issues of *Nature*.



**Fig. 10** *a*, Reconstruction of a penetration through the striate cortex about 1 mm from the 17–18 border, near the occipital pole of a spider monkey called George. To the left of the figure, the lines indicate receptive-field orientations of cells in the columns traversed; each line represents one or several units recorded against a rich unresolved background activity. Arrows indicate reversal of directions of shifts in orientation<sup>32</sup>. *b*, Graph of stimulus orientation in degrees versus distance along the electrode track in millimetres, in the experiment shown in *a*. Vertical is taken as 0°, clockwise is positive and anticlockwise negative.

## Orientation columns

What our three simultaneously recorded cells, numbers 3009, 3010 and 3011, mapped out on the overhead sheet in September 1958 with their parallel orientation axes and separate but overlapping field positions, were telling us was that neighbouring cells have similar orientations but slightly different receptive-field positions. We of course knew about Mountcastle's somatosensory columns, and we began to suspect that cells might be grouped in the striate cortex according to orientation; but to prove it was not easy (Fig. 9).

Our first indication of the beauty of the arrangements of cell groupings came in 1961 in one of our first recordings from the striate cortex of monkey, a spider monkey named George. In one penetration, which went into the cortex at an angle of about 45° and was 2.5 mm long, we were struck right away by something we had only seen hints of before. As the electrode advanced, the orientations of successively recorded cells progressed in small steps of about 10° for every advance of 50 μm. We began the penetration about 8:00 p.m.; 5 hours later we had recorded 53 successive orientations without a single large jump in orientation (Fig. 10). During the entire time, in which I wielded the slide projector and Torsten mapped the fields, neither of us moved from our seats. Fortunately, our fluid intake that day had been modest. Though I have shown this illustration many times, so far only Francis Crick has asked why there was no interruption in layer 4C, where according to dogma the cells are not orientation-specific. The answer is that I do not know.

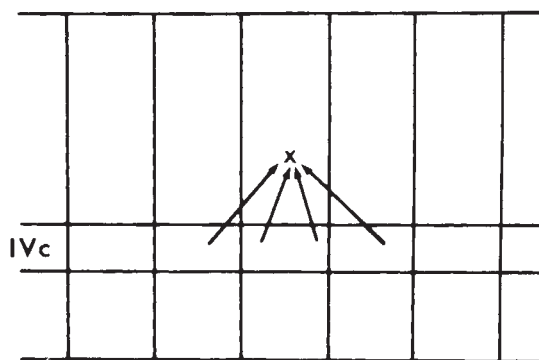
In the cat we had had occasional suggestions of similar orderliness, and so we decided to address directly the problem of the shape and arrangement of the groupings<sup>18</sup>. By making

several closely spaced oblique parallel penetrations, we convinced ourselves that the groupings were really columns in that they extended from the surface to white matter and had walls that were perpendicular to the layers. We next made multiple close-spaced penetrations, advancing the electrode just far enough in each penetration to record one cell or a group of cells. To map a few square millimetres of cortex this way required 50 to 100 penetrations, each of which took 10 to 15 minutes. We decided it might be better to change careers, perhaps to chicken farming. But although the experiments were by our standards exhausting they did succeed in showing that orientation columns in the cat are not generally pillars but parallel slabs that intersect the surface as either straight parallel stripes or swirls.

Reversals in direction of orientation shift (Fig. 10*a*) are found in most penetrations. They occur irregularly, on the average about once every millimetre, and not at any particular orientation such as vertical or horizontal. We still do not know how to interpret them. Between reversals the plots of orientation against electrode position are remarkably linear<sup>19</sup>. Once, to exercise a new programmable calculator, I actually determined the coefficient of linear correlation of such a graph. It was 0.998, which I took to mean that the line must be very straight indeed.

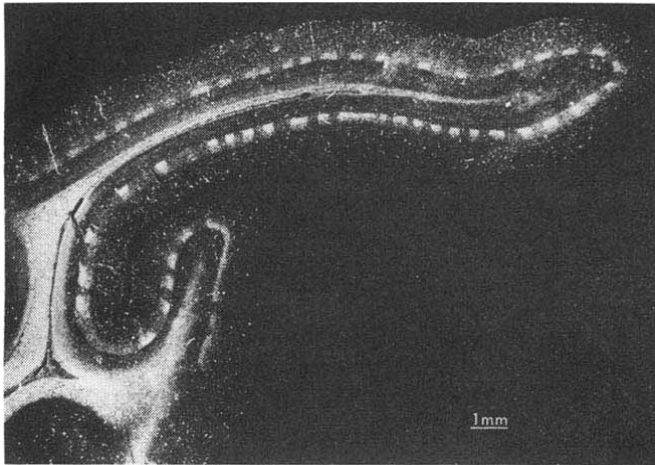
For some years we had the impression that regular sequences like the one shown in Fig. 10 are rare—that most sequences are either more or less random or else the orientation hovers around one angle for some distance and then goes to a new angle and hovers there. Chaos and hovering do occur but they are exceptional, as are major jumps of 45° to 90°. It took us a long time to realize that regularity is the rule, not the exception, probably because we did not begin making very oblique or tangential penetrations until the mid-1970s. For these experiments to be successful requires electrodes coarse enough to record activity throughout a penetration, and not simply every 100 μm or so. Such electrodes look less aesthetically pleasing, a fact that I think has happily tended to keep down the competition.

Our attempts to learn more about the geometry of orientation columns in the monkey by using the 2-deoxy-D-glucose technique<sup>20</sup> suggest that iso-orientation lines form a periodic pattern but are far from straight, being full of swirls and interruptions. Experiments done since then<sup>21</sup> suggest that the deoxyglucose is probably also labelling the cytochrome blobs (see below). Similar work in the tree shrew by Humphrey *et al.*<sup>22</sup> has shown



**Fig. 11** Wiring of a binocular cell in a layer above (or below) layer 4C. In the macaque, the bulk of the afferents to the striate cortex from the lateral geniculate body, themselves monocular, are strictly segregated by eye affiliation in layer 4C, and thus the cells in this layer are strictly monocular. A cell outside layer 4C (X) receives its input connections, directly or indirectly by one or more synapses, from cells in layer 4C (to some extent also, perhaps, from layers 4A and 6). Cells in layer 4C will be more likely to feed into X the closer they are to it; consequently X is likely to be binocular, dominated by the eye corresponding to the nearest patch in 4C. The degree of dominance by that eye is greater, the closer X is to being centred in its ocular dominance column, and cells near a boundary may be roughly equally influenced by the two eyes (Fig. 12 in ref. 29).





**Fig. 12** Dark-field autoradiograph of the striate cortex in an adult macaque in which the ipsilateral eye had been injected with tritiated proline-fucose 2 weeks previously. Labelled areas show white. The section passes in a plane roughly perpendicular to the exposed surface of the occipital lobe and to the buried part immediately beneath (roughly, through the arrow of Fig. 9a). In all, about 56 labelled patches can be seen (Fig. 22 in ref. 29).

a much more regular pattern, and Stryker, Wiesel and I have seen more regularity in the cat (unpublished data). Both tree shrew and cat lack the cytochrome blobs.

### Ocular dominance columns

A major finding in our 1959 and 1962 papers<sup>10,12</sup>, besides the orientation selectivity, was the presence in the striate cortex of a high proportion of binocular cells. Since recordings from the lateral geniculate body had made it clear that cells at that stage are for all practical purposes monocular, this answered the question of where, in the retinogeniculocortical pathway, cells first received convergent input from the two eyes. More interesting to us than the mere binocularity was the similarity of a given cell's receptive fields in the two eyes in size, complexity, orientation and position. Presumably this forms the basis of the fusion of the images in the two eyes. It still seems remarkable that a cell should not only be wired with the precision necessary to produce complex or hypercomplex properties, but should have a duplicate set of such connections, one from each eye.

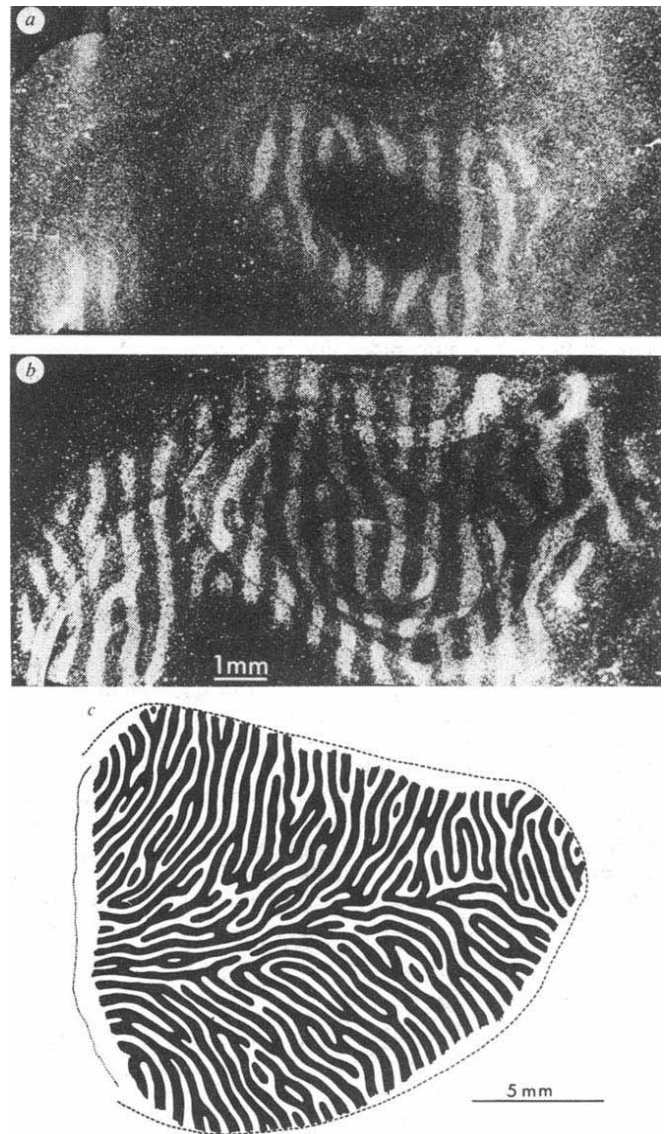
Although the optimum stimulus is the same for the two eyes, the responses evoked are not necessarily equal; for a given cell one eye is often consistently better than the other. It is as if the two sets of connections were qualitatively similar, but, for many cells, different in density. We termed this relative effectiveness of the two eyes 'eye preference' or 'relative ocular dominance'.

In the macaque it was evident from the earliest experiments that neighbouring cells have similar eye preferences. In vertical penetrations the preference remains the same all the way through the cortex. In layer 4C the cells are monocular, and here any cell is monopolized by the eye that merely dominates the cells in the layers above and below. In penetrations that run parallel to the layers eye preference alternates, with shifts roughly every 0.5 mm. The conclusion is that the terminals from cells of the lateral geniculate distribute themselves in layer 4C according to eye of origin, in alternating patches about 0.5 mm wide. In the layers above and below layer 4, horizontal and diagonal connections lead to a mixing that is incomplete, so that a cell above a given patch is dominated by the eye supplying that patch but receives subsidiary input from neighbouring patches (Fig. 11).

The geometry of these layer-4 patches was finally determined by several independent anatomical methods, the first of which<sup>23</sup> was the Nauta method and its modifications for staining terminals worked out first by Fink and Heimer and then by a most able and energetic research assistant, Janet Wiitanen. By making small lesions in single geniculate layers, we were able to

see the patchy distribution of degenerating terminals in layer 4, which, in a face-on view, takes the form not of circumscribed patches but of parallel stripes. We also showed that the ventral (magnocellular) pair of layers projects to the upper half of layer 4C (subsequently called 4C $\alpha$  by Jennifer Lund), whereas the dorsal four layers project to the lower half (4C $\beta$ ), and that the line of Gennari (4B), once thought to receive the strongest projection, is actually almost bereft of geniculate terminals.

While the Nauta studies were still in progress, we read Bernice Grafstein's report that radioactive label injected into



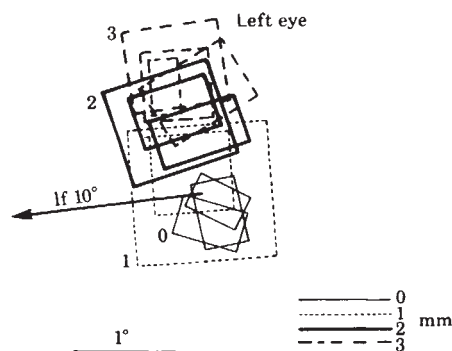
**Fig. 13** Autoradiographs from the same (normal) animal as in Fig. 12, but from the hemisphere contralateral to the injected eye (dark field). *a*, A section tangential to the exposed dome-like surface of the occipital lobe, just grazing layer 5, which appears as an oval, surrounded by layer 4C which appears as a ring containing the labelled parallel bands, which appear light against the dark background. *b*, A composite made by cutting out layer 4C from a number of parallel sections such as the one shown in *a* and pasting them together to show the bands over an area some millimetres in extent. *c*, Reconstruction of layer 4C ocular dominance columns over the entire exposed part of area 17 in the right occipital lobe shown in Fig. 9a. The line on the left represents the midsagittal plane, where the cortex bends around. The dashed reversed c-shaped curve is the 17-18 border, whose apex, to the extreme right, represents the fovea. Every other column has been blacked in to exhibit the twofold nature of the set of subdivisions. Note the relative constancy of column widths.



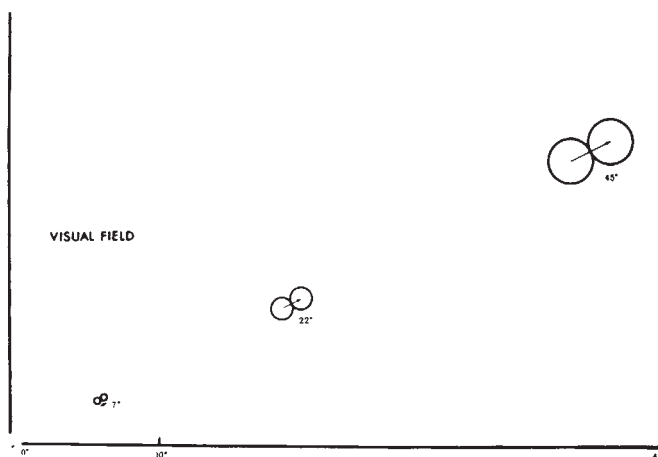
the eye of a rat could be detected in the contralateral visual cortex, as though transneuronal transport had taken place in the geniculate<sup>24</sup>. (The rat retinogeniculocortical pathway is mainly crossed.) It occurred to us that if we injected the eye of a monkey we might be able to see autoradiographic label in area 17. We tried it, but could see nothing. Soon after, while visiting Ray Guillery in Wisconsin, I saw some amino acid transport autoradiographs which showed nothing in light field but in which label was obvious in dark field. I rushed back, we got out our slides, borrowed a dark-field condenser and found beautiful alternating patches throughout all the binocular part of area 17<sup>25</sup> (Fig. 12). This method allowed us to reconstruct ocular dominance columns over much wider expanses than could be mapped with the Nauta method (Fig. 13). It led to a study of the pre- and postnatal visual development of ocular dominance columns and the effects of visual deprivation on the columns, which Torsten Wiesel will describe.

## Relationship between columns, magnification and field size

To me the main pleasures of doing science are in getting ideas for experiments, doing surgery, designing and making equipment, and above all the rare moments in which some apparently isolated facts click into place like a Chinese puzzle. When a collaboration works, as ours has, the ideas and the clicking into place often occur simultaneously or collaboratively; usually neither of us has known (or cared about) which of us originally produced an idea, and sometimes one idea has occurred to one of us, only to be forgotten and later resurrected by the other. One of the most exciting moments was the realization that our orientation columns, extending through the full thickness of the cat cortex, contain just those simple and complex cells (later we could add the hypercomplex) that our hierarchical schemes had proposed were interconnected<sup>10</sup>. This gave the column a meaning: a little machine that takes care of contours in a certain orientation in a certain part of the visual field. If the cells of one set are to be interconnected, and to some extent isolated from neighbouring sets, it makes obvious sense to gather them together. As Lorente de Nó showed<sup>26</sup>, most of the connections in the cortex run up and down; lateral or oblique connections tend to be short (mostly limited to 1–2 mm) and less rich. These ideas were not entirely new since Mountcastle had clearly enunciated the principle of the column as an independent unit of function. What was new in the visual cortex was a clear function for the columns, the transformation of information



**Fig. 14** Receptive-field drift. Receptive fields mapped during on oblique, almost tangential, penetration through the striate cortex. A few fields were mapped along each of four 100- $\mu$ m segments, spaced at 1-mm intervals. These four groups of fields are labelled 0, 1, 2, and 3. Along any one of these 100- $\mu$ m segments no systematic progression of receptive fields could be seen; any such movement was obscured by the random scatter. But from one segment to the next there was a clear movement: each new set of fields was slightly above the other in the visual field, as predicted from the direction of movement of the electrode and from the topographic map of visual fields onto the cortex. Roughly a 2-mm movement through cortex was required to displace the fields from one region to an entirely new region (Fig. 2 in ref. 28).



**Fig. 15** Variation of receptive-field drift with eccentricity. The diagram represents one quadrant of the field of vision, and the circles represent aggregate receptive fields—the territory collectively occupied by receptive fields of cells encountered in a micro-electrode penetration perpendicular to the cortical surface. Each pair of circles illustrates the movement in aggregate receptive fields accompanying a tangential movement along the cortex of 1–2 mm. Both the displacement and the aggregate field size vary with distance from the fovea (eccentricity), but they do so in parallel fashion. Close to the fovea the fields are tiny, but so is the displacement accompanying a 1–2-mm movement along the cortex. (At 0° eccentricity, displacement and aggregate field size are both too small to be reproduced in this figure.) The greater the distance from the fovea, the greater the two become, but they continue to remain roughly equal<sup>28</sup>.

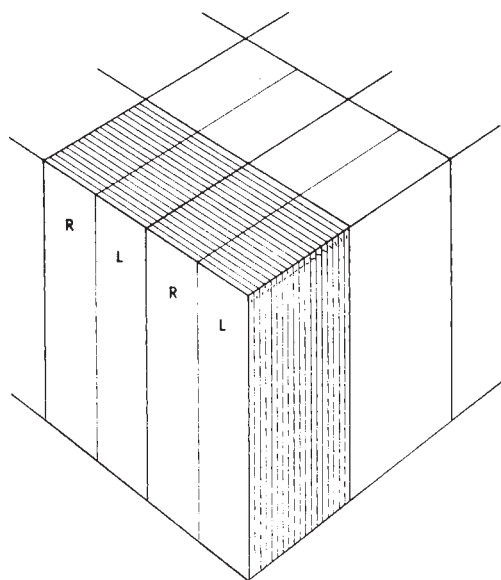
from circularly symmetric form to orientation-specific form, and the stepwise increase in complexity.

A similar argument applies to the ocular dominance columns, a pair of which constitutes a machine for combining inputs from the two eyes—combining, but not completely combining, in a peculiar grudging way for reasons still not at all clear, but probably related in some way to stereopsis. (Whatever the explanation of the systematically incomplete blending, it will have to take into account the virtual but not complete absence of dominance columns in squirrel monkeys.) If the eyes are to be kept to some extent functionally separate, it is economical of connections to pack together cells of a given eye preference.

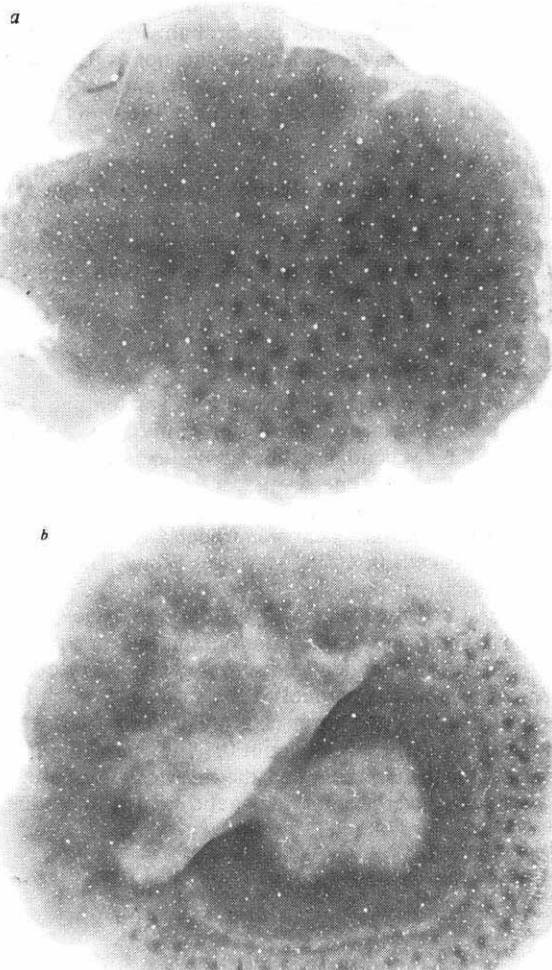
To my mind our most aesthetically attractive and exciting formulation has been the hypercolumn and its relation to magnification. The idea grew up gradually, but took an initial spurt as a result of a question asked by Werner Reichardt during a seminar that I gave in Tübingen. I had been describing the ordered orientation sequences found in monkeys like George, when Werner asked how one avoided the difficulty arising from the fact that as you move across the cortex, visual field position is changing in addition to orientation. Could this mean that if you looked closely you would find, in one small part of the visual field, only a small select group of orientations represented? The question seemed silly (at first), and I explained that in any one part of the visual field all orientations are represented, in fact probably several times over. Afterwards the question nagged me. There must be more to it than that. We began to put some seemingly isolated facts together. The visual fields map systematically onto the cortex but the map is distorted: the fovea is disproportionately represented, with 1 mm about equivalent to 1/6° of visual field. As one goes out in the visual field the representation falls off logarithmically, as Daniel and Whitteridge had shown<sup>27</sup>, so that in the far periphery the relationship is more like 1 mm = 6°. Meanwhile the average size of receptive fields grows from centre of gaze to periphery. This is not unexpected when one considers that in the fovea our acuity is much higher than in the periphery. To do the job in more detail takes more cells, each looking after a smaller region; to accommodate the cells takes more cortical surface area. I had always been surprised that the part of the cortex

representing the fovea is not obviously thicker than that representing the periphery: surprised, I suppose, because in the retina near the fovea, the ganglion is, in fact, many times thicker than in the periphery. The cortex must be going out of its way to keep its uniformity by devoting to the detailed tasks more area rather than more thickness.

We decided to look more carefully at the relationship between receptive field size and area of cortex per unit area of visual field<sup>28</sup>. When an electrode pushed vertically through the cortex encounters a hundred or so cells in traversing the full thickness, the receptive fields vary to some extent in size, and in a rather random way in position, so that the hundred maps when superimposed cover an area several times that of an average receptive field. We call this the 'aggregate receptive field' for a particular point on the cortex. On making a penetration parallel to the surface, a gradual drift in field positions is superimposed on the random staggering, in a direction dictated by the topographic map (Fig. 14). We began to wonder whether any law connected the rate of this drift in aggregate position and the size of the fields. It turned out that for layers 2 and 3 a movement of about 2 mm across the cortex is just sufficient to produce a displacement, in the visual field, out of the region where one started and into an entirely new region. This held across the entire striate cortex (and consequently over the whole visual field). In the fovea the displacement was tiny and so were the fields. As one went out, both increased in size, in parallel fashion (Fig. 15). Now things seemed to mesh. George and other monkeys had taught us that a 1–2-mm movement across the cortex is accompanied by an angular shift in receptive field orientation of 180° to 360°, more than one full complement of orientations. We have termed such a set of orientation columns (180°) a hypercolumn. Meanwhile, the ocular dominance shifts back and forth to take care of both eyes every millimetre—a hypercolumn for ocular dominance. Thus in 1 or 2 mm<sup>2</sup> there seems to exist all the machinery necessary to look after everything the visual cortex is responsible for, in a certain small part of the visual world. The machines are the same everywhere; in



**Fig. 16** Model of the striate cortex, to show roughly the dimensions of the ocular dominance slabs (L and R) in relation to the orientation slabs and the cortical thickness. Thinner lines separate individual columns: thicker lines demarcate the two pairs of ocular dominance columns and two sets of orientation columns. The placing of these hypercolumn boundaries is arbitrary: one could as well begin the orientation hypercolumn at horizontal or at any of the obliques. The decision to show the two sets of columns as intersecting at right angles is also arbitrary, since there is at present no evidence of the relationship between the two sets. For convenience, the slabs are shown as plane surfaces, but whereas the dominance columns are indeed more or less flat, the orientation columns are not known to be so, and when viewed from above, they may have the form of swirls (Fig. 27 in ref. 29).



**Fig. 17** Tangential sections (cytochrome oxidase stain) through the visual cortex of the squirrel monkey. The sections pass through the 17–18 border, which runs obliquely in the figure, with area 17 below and to the right and 18 above and to the left. (D.H.H. and M.S.L., unpublished data). *a*, The section passes through layer 3, and the blobs can be seen easily in area 17. *b*, The section is tangential to layer 5, where the blobs can again be seen, though faintly; these lie in register with the upper-layer blobs.

some parts the information on which they do their job is less detailed, but covers more visual field (Fig. 16).

Uniformity is surely a huge advantage in development, for genetic specifications need only be laid down for a 1–2-mm block of neural tissue, together with the instruction to make a thousand or so.

We could have called the entire machine a hypercolumn, but we did not. The term as we define it refers to a complete set of a given column. I mention this because in terminology, too, uniformity has some obvious advantages. Perhaps one could use 'module' to refer to the complete machine.

There are three qualifications to all of this. (1) I do not mean to imply that there need really be 2,000 separate definable entities. It need not matter whether one begins a set of orientation columns at vertical, horizontal or any one of the obliques; the decision is arbitrary. One requires two dominance columns, a left and a right, and it makes no difference which one begins with. (In fact, as will become apparent when I discuss cytochrome blobs, it now looks as though the blocks of tissue may really be discrete, to a degree that we could not have imagined 2 years ago.) (2) Because receptive fields are larger and connections longer in layers 5 and 6, compared with those of layers 2 and 3, a module defined for the deeper layers would be somewhat larger than 2 × 2 mm. In this sense, to think of modules as small chunks of cortex was an oversimplification. (3) There may well be some differences in cortical machinery



between the centre and periphery of the visual field. Colour vision and stereopsis, for example, probably decline in importance far out in the visual fields. I say this not to be obsessively complete but because in the next few years someone will probably find some difference and pronounce the general concept wrong. It may of course be wrong, but I hope it will be for interesting reasons.

The retina must be nonuniform if it is to do a more detailed job in the centre. To have more area devoted to the centre than to the periphery is not an option open to it, because it is a globe. Were it anything else the optics would be awkward and the eye could not rotate in its socket.

## New developments

A few years ago, in a Ferrier Lecture<sup>29</sup>, Torsten and I ended by saying that the striate cortex was probably, in broad outline, understood. This was done deliberately: one did not want the well to dry up. When one wants rain the best strategy is to leave raincoat and umbrella at home. So the best way to guarantee future employment was to declare the job finished. It certainly worked. In 1978, Margaret Wong-Riley observed regular intermittent puff-like densities in the upper layers of monkey striate cortex stained for the enzyme cytochrome oxidase, and 2 years ago Anita Hendrickson and her group and our laboratory independently discovered that if cytochrome-stained cortex was cut parallel to the surface through layer 3 these densities took the form of a polka-dot pattern of dark blobs quasi-regularly spaced about 0.5 mm apart (Fig. 17)<sup>21,30</sup>. It is as if the animal's brain had the measles. The pattern has been seen with several other enzymatic stains, suggesting that

either the activity or the machinery is different in the blob regions. The pattern has been found in all primates examined, including humans, but not, so far as I know, in any non-primates. In the macaque the blobs are clearly lined up along ocular dominance columns<sup>21</sup>. Over the past year Margaret Livingstone and I have shown that the cells in the blobs lack orientation selectivity, resembling, at least superficially, cells of layer 4C<sup>31</sup>. They are selectively labelled after large injections of radioactive proline into the lateral geniculate body, so it is clear that their inputs are not identical to the inputs to the rest of layers 2 and 3. Thus an entire system has opened up, whose existence we were previously unaware of and whose anatomy and functions we do not yet understand. We are especially anxious to learn what, if any, relationship exists between the cytochrome blobs and the orientation columns.

Things are at an exciting stage. There is no point leaving the umbrella home; it is raining, and raining hard.

I thank the Eye Institute of the National Institutes of Health, the US Air Force, the Klingenstein Fund, and the Rowland Foundation for their generous support of our research; also, the Faculty of Harvard University for tolerating such a truculent colleague. I thank many research assistants who have helped Torsten Wiesel and me over the past 22 years, especially Jane Chen, Janet Wiitanen, Bea Storai, Jaye Robinson, Martha Egan, Joan Weisenbeck, Karen Larson, Sharon Mates, Debra Hamburger, Yu-Wen Wu, Sue Fenstermaker, Stella Chow, Sarah Kennedy, Maureen Packard and Mary Nastuk. For photographic assistance I thank Sandra Spinks, Carolyn Yoshikami and Marc Peloquin, in electronics and computers David Freeman, and Sheila Barton, Pat Schubert and Olivia Brum for secretarial help.

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## ARTICLES

# Mafic segregations in ophiolite mantle sequences

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*Mafic segregations of picritic composition and high-temperature-high-pressure mineralogy are described from the mantle sequences of ophiolites and show similarities with parental magma to oceanic tholeiite and have escaped significant fractionation and re-equilibration due to ascent through an unperturbed geothermal gradient. Such conditions are uncharacteristic of mature spreading centres and indicate that the host ophiolites were generated during incipient rifting.*

A HITHERTO little documented component of ophiolite complexes are mafic segregations found in association with ultramafic tectonites (Fig. 1). The host ultramafics are commonly plagioclase- or spinel-bearing lherzolites which occur as pods (km-size) surrounded by harzburgite; as intercalations (usually <1 km thick) parallel-banded with harzburgite tectonite fabrics, or as a basal zone to the ophiolite mantle sequence (usually <<1 km thick). Within the lherzolites, the

mafic segregations form thin discontinuous layers, pods or lenses (usually <10 cm thick) in parallel orientation with lherzolite tectonite fabrics or alternatively, discordant veins and dykelets. At higher levels in the mantle sequence the mafic segregations are similarly disposed but are often hosted by harburgites. The segregations may exhibit granoblastic, mylonitic, schistose or gneissose textures indicative of sub-solidus deformation, or alternatively interlocking mineral

textures suggestive of magmatic crystallization. Most segregations are of basaltic bulk composition with a mineralogy dominated by pyroxene  $\pm$  garnet  $\pm$  amphibole  $\pm$  plagioclase  $\pm$  spinel  $\pm$  olivine. Many of the parageneses indicate high pressures and temperatures of formation normally realized at several tens of kilometres depth in the Earth's mantle. Their presence in ophiolites, which are generally <15 km thick, is therefore problematic. The textures, chemistry and mineralogy of mafic segregations from five ophiolite localities (Newfoundland, Greece, Yugoslavia, Scotland and England) are considered here and their probable origins discussed.

## Occurrences of mafic segregations

In western Newfoundland, the Bay of Islands Complex and White Hills Peridotite represent transported Cambro-Ordovician ophiolites<sup>1</sup>. In the Bay of Islands most of the mantle tectonites are harzburgitic but at North Arm Mountain and Table Mountain, where the basal contact is exposed, 500 m of spinel lherzolite overlies a metamorphic sole. A lherzolite mylonite (<50 m thick) forms the very base of the Table Mountain ophiolite within which are found amphibole + phlogopite + garnet + spinel intercalations forming discrete layers (<10 cm thick)<sup>2,3</sup>. Similarly, within the White Hills Peridotite,

Jamieson<sup>4</sup> describes lherzolite and pyroxenite interlayered with harzburgite, and thin layers of pargasite parallel with and deformed by a 30 m basal peridotite mylonite. Jamieson emphasizes that the amphibole-bearing peridotites are hydrated lherzolites rather than being interleaved peridotite and aureole-derived amphibolite (Table 1: M1, P1).

The Mesozoic ophiolites extending through Greece, Albania and Yugoslavia include both lherzolite and dunite-harzburgite varieties defining the boundary between the western and eastern styles of Mediterranean Alpine-type peridotites respectively<sup>5-7</sup>. The Pindos and Othris ophiolites of Greece possess basal mantle sequences containing plagioclase lherzolites and in Othris these are associated with gabbroic schlieren<sup>8-10</sup>. The schlieren comprise variably rodingitized pyroxene + plagioclase + olivine assemblages (3–50 cm wide) which Menzies interprets as partial melts<sup>8</sup> (Table 1: M2, P3). In Yugoslavia the lherzolitic ophiolites of the Outer Dinarides, including the Borja, Krivaja and Zlatibor massifs, all possess basal high-grade mafic intercalations in the aegirite and eclogite facies<sup>7,11,12</sup> (Table 1: M3, P3).

In Britain, the Ballantrae Igneous Complex of southwestern Scotland is a dismembered Cambro-Ordovician ultramafic-mafic sequence. Most components of an ophiolite are present<sup>13</sup> as well as a high temperature metamorphic sole<sup>14-16</sup>. The ultramafics are highly serpentinized and most appear to have

**Table 1** Chemistry, CIPW norms and mineral parageneses of mafic segregations and peridotite hosts from the selected ophiolite mantle sequences

| Wt %                               | Dyke in mantle xenolith |                 |       | Mafic segregations within ophiolites |       |        |       |       | Peridotite hosts |        |        |       |       |
|------------------------------------|-------------------------|-----------------|-------|--------------------------------------|-------|--------|-------|-------|------------------|--------|--------|-------|-------|
|                                    | Picrite                 | Garnet aegirite |       | M1                                   | M2    | M3     | M4    | M5    | P1               | P2     | P3     | P4    | P5    |
| SiO <sub>2</sub>                   | 46.86                   | 41.00           | 43.93 | 43.98                                | 44.87 | 43.42  | 39.19 | 44.26 | 44.30            | 40.32  | 43.67  | 41.46 | 44.72 |
| TiO <sub>2</sub>                   | 0.72                    | 2.40            | 1.00  | 0.85                                 | 0.09  | 0.07   | 1.68  | 0.52  | 0.27             | —      | 0.05   | 0.32  | 0.18  |
| Al <sub>2</sub> O <sub>3</sub>     | 14.59                   | 13.00           | 10.79 | 12.99                                | 10.69 | 14.30  | 11.87 | 13.95 | 2.08             | 0.53   | 2.97   | 7.12  | 3.52  |
| Fe <sub>2</sub> O <sub>3</sub>     | —                       | 0.56            | —     | —                                    | —     | 2.79   | 4.83  | —     | 1.18             | —      | 2.34   | 2.96  | —     |
| FeO                                | 10.30*                  | 9.94            | 9.06* | 10.71*                               | 2.72* | 8.38   | 16.48 | 4.49* | 7.42             | 8.91*  | 7.88   | 6.68  | 8.23* |
| MnO                                | 0.12                    | 0.14            | 0.14  | 0.21                                 | 0.07  | 0.20   | 0.51  | 0.08  | 0.14             | 0.15   | 0.18   | 0.10  | 0.14  |
| MgO                                | 15.98                   | 17.80           | 17.73 | 20.38                                | 14.11 | 15.03  | 18.01 | 23.04 | 38.20            | 49.01  | 39.35  | 24.21 | 40.48 |
| CaO                                | 9.29                    | 11.05           | 15.23 | 8.51                                 | 16.46 | 13.67  | 5.28  | 12.94 | 3.11             | 0.88   | 2.75   | 9.46  | 2.03  |
| Na <sub>2</sub> O                  | 1.94                    | 1.53            | 1.09  | 1.13                                 | 1.77  | 1.02   | 0.22  | 0.46  | 0.31             | 0.16   | 0.15   | 0.20  | 0.18  |
| K <sub>2</sub> O                   | 0.10                    | 0.40            | 0.20  | —                                    | 0.03  | 0.05   | —     | 0.11  | 0.18             | 0.05   | —      | 0.07  | 0.07  |
| P <sub>2</sub> O <sub>5</sub>      | —                       | —               | 0.01  | 0.06                                 | —     | —      | 0.07  | —     | 0.03             | —      | —      | 0.01  | —     |
| H <sub>2</sub> O                   | —                       | 1.96            | —     | 3.01                                 | —     | 1.46   | 1.37  | —     | 1.60             | —      | 1.05   | 6.85  | —     |
| Total                              | 99.90                   | 99.78           | 99.18 | 101.83                               | 99.81 | 100.39 | 99.51 | 99.85 | 98.82            | 100.01 | 100.39 | 99.44 | 99.55 |
| 100 Mg/Mg + Fe                     | 73.4                    | 75.2            | 77.7  | 77.2                                 | 90.2  | 71.0   | 60.7  | 90.1  | 88.9             | 90.7   | 87.5   | 82.2  | 89.8  |
| CaO/Al <sub>2</sub> O <sub>3</sub> | 0.64                    | 0.85            | 1.41  | 0.66                                 | 0.86  | 0.96   | 0.45  | 0.93  | 1.50             | 1.66   | 0.93   | 1.33  | 0.58  |
| <b>CIPW Norm†</b>                  |                         |                 |       |                                      |       |        |       |       |                  |        |        |       |       |
| C                                  | —                       | —               | —     | —                                    | —     | —      | 2.1   | —     | —                | —      | —      | —     | —     |
| Or                                 | 0.6                     | —               | —     | —                                    | —     | —      | —     | —     | 1.1              | —      | —      | 0.4   | 0.4   |
| Ab                                 | 16.4                    | —               | —     | 9.6                                  | —     | —      | 1.9   | —     | 2.6              | —      | 1.3    | 1.7   | 1.5   |
| An                                 | 30.8                    | 27.4            | 24.0  | 30.4                                 | 45.7  | 34.3   | 25.7  | 35.7  | 3.8              | 0.6    | 7.4    | 18.3  | 8.6   |
| Lc                                 | —                       | 1.9             | 0.9   | —                                    | 0.1   | 0.2    | —     | 0.5   | —                | —      | —      | —     | —     |
| Ne                                 | —                       | 7.0             | 5.0   | —                                    | 8.1   | 4.7    | —     | 2.1   | —                | 0.7    | —      | —     | —     |
| Di                                 | 12.3                    | 20.1            | 32.3  | 9.2                                  | 22.3  | 26.6   | —     | 20.9  | 9.1              | —      | 4.9    | 22.8  | 1.2   |
| Hy                                 | 3.4                     | —               | —     | 4.1                                  | —     | —      | 19.0  | —     | 15.5             | —      | 14.0   | 3.5   | 19.4  |
| Ol                                 | 35.0                    | 36.1            | 31.6  | 43.9                                 | 21.0  | 32.5   | 45.6  | 39.1  | 64.5             | 98.4   | 71.4   | 44.9  | 68.2  |
| Il                                 | 1.4                     | 4.6             | 1.9   | 1.6                                  | 0.2   | 0.1    | 3.2   | 1.0   | 0.5              | —      | 0.1    | 0.6   | 0.3   |
| Ap                                 | —                       | —               | —     | 0.1                                  | —     | —      | 0.2   | —     | 0.1              | —      | —      | —     | —     |
| <b>Main paragenesis</b>            |                         |                 |       |                                      |       |        |       |       |                  |        |        |       |       |
| Amphibole                          |                         | x               | x     | x                                    |       |        | x     | x     | x                |        |        |       | x     |
| Clinopyroxene                      | Glass                   | x               | x     | x                                    | x     | x      | x     | x     | x                |        | x      | x     | x     |
| Orthopyroxene                      |                         | x               |       | x                                    | x     | x      |       | x     | x                | x      | x      | x     | x     |
| Olivine                            |                         | x               | x     | x                                    | x     | x      |       | x     | x                | x      | x      | x     | x     |
| Plagioclase                        |                         |                 |       | x                                    | x     |        |       | x     |                  | x      |        |       | x     |
| Spinel                             |                         | x               | x     | x                                    |       |        | x     | x     | x                | x      | x      | x     | x     |
| Garnet                             |                         | x               |       | x                                    |       | x      | x     |       |                  |        |        |       |       |

Shown for comparison are: A, possible parental magma (picrite) to MORB: basaltic melt in equilibrium with OPX-OL at 20 kbar (sample 519-16, ref. 47); B, hydrous garnet aegirite dyke cross-cutting spinel lherzolite from the Lherz massif, Ariège, France (sample ARG-2, ref. 29); C, spinel-olivine-amphibole clinopyroxenite dyke margin in spinel lherzolite xenolith from Lake Eacham, north Queensland, Australia (sample LE76-1P, ref. 48). M1: Amphibole-garnet aegirite in serpentinized lherzolite, Bay of Islands Complex, West Newfoundland<sup>3</sup>. M2: Mafic segregation in the dunite-harzburgite series (P2), Othris ophiolite, Greece<sup>8</sup>. M3: 'Eclogite' vein in lherzolite (L3), Zlatibor ophiolite, Yugoslavia<sup>12</sup>. M4: Garnet-pyroxene-amphibole-spinel rock associated with serpentinized peridotites, Ballantrae ophiolite, southwestern Scotland<sup>16</sup>. M5: Recrystallized basic band within anhydrous recrystallized lherzolite (L5), Lizard complex, southwestern England<sup>18</sup>. P1: Amphibole lherzolite, White Hills peridotite, northwestern Newfoundland<sup>4</sup>. P2: Harzburgite from the dunite-harzburgite series, Othris ophiolite, Greece<sup>8</sup>. P3: Lherzolite tectonite, Zlatibor ophiolite, Yugoslavia<sup>12</sup>. P4: Serpentinized wehrlite, Ballantrae ophiolite, southwestern Scotland<sup>17</sup>. P5: Anhydrous recrystallized lherzolite, Lizard complex, southwestern England<sup>18</sup>.

\* Total Fe as FeO.

† All iron calculated as ferrous in norm.

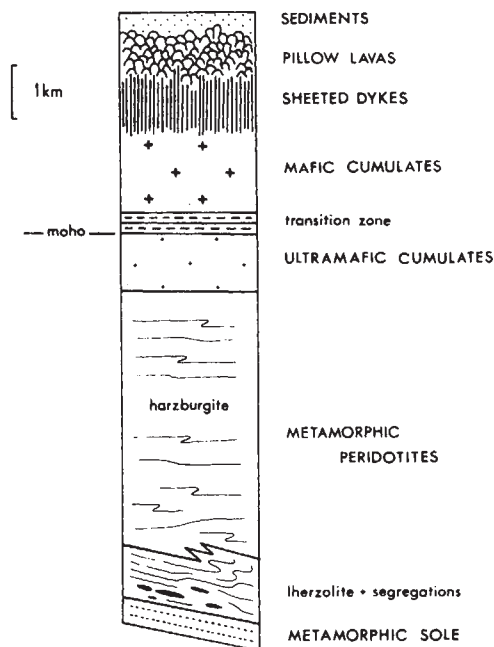


Fig. 1 Idealized cross-section of a typical ophiolite showing internal components, including positions of lherzolites and mafic segregations in the mantle sequence (metamorphic peridotites) and basal metamorphic sole.

been harzburgites, but interbanded spinel wehrlites and clinopyroxenites occur within them<sup>15,17</sup>. In addition, thin layers and pods of aegirite are preserved within a basal lherzolite unit and at the top of the metamorphic sole<sup>15-17</sup> (Table 1: M4, P4). The Lizard Complex of southwestern England is an ultramafic-mafic sequence of probable Lower Palaeozoic age comprising primary, recrystallized and hydrated lherzolites<sup>18</sup>, supposed metamorphic sole<sup>19</sup> and a topmost gabbro-dolerite unit<sup>20</sup>. At the margins of the complex finely foliated recrystallized anhydrous lherzolite contains thin (0.5–2 cm) planar basic bands<sup>18</sup> (Table 1: M5, P5).

Many other ophiolites possess mafic segregations associated with mantle peridotites: these include the Tinaquillo peridotite, Venezuela<sup>21</sup>; the Red Hills ultramafic intrusion, New Zealand<sup>22</sup>; the Mount Albert ultramafic complex, Canada<sup>23</sup> and the Trinity peridotite, California<sup>24</sup>. Some ophiolites have mantle sequences apparently dominated by lherzolites: recent work on the Xigaze ophiolite of southern Tibet shows that the ultramafic unit is poor in residual harzburgite and dunite but is dominated by chrome diopside-rich harzburgites (that is, lherzolites)<sup>25</sup>.

The above examples show that the presence of mafic segregations and lherzolite in ophiolites is not uncommon, especially as a minor basal component of mantle tectonites which are otherwise dominated by harzburgite and dunite. In many cases the apparent absence of the association can more probably be attributed to tectonic removal or to extensive serpentinization inhibiting its recognition rather than to primary omission. However, certain ophiolites appear to contain only very rare lherzolites and related segregations: peridotites of the Samail ophiolite in Oman predominantly comprise 9–12 km of harzburgite and dunite<sup>26</sup>; the same is true for the Troodos Complex of Cyprus, although the base of this ophiolite is not exposed<sup>27</sup>.

It is important to emphasize that the mafic layer-lherzolite association has previously been considered diagnostic of the lherzolitic variety of the Alpine-type peridotites exemplified by the ultramafic massifs of the Western Mediterranean (for example, Lherz<sup>28,29</sup>, Ronda<sup>30</sup>, Beni Bouchera<sup>31</sup>). These high-temperature-high-pressure bodies are interpreted as diapirs of relatively undepleted continental or deep oceanic mantle. A crustal mafic unit, so typical of ophiolites, is absent or at best poorly developed in this subtype. Instead, mafic segregations tend to be dispersed throughout the body of lherzolite. On the

other hand, ophiolites have been interpreted as the harzburgitic, low-temperature-low-pressure variety of the Alpine-type peridotites typical of the ultramafic-mafic massifs of the Eastern Mediterranean (for example, Troodos<sup>32</sup>, Antalya<sup>33</sup>, Baer-Bassit<sup>34</sup>). These massifs possess well-developed mafic crustal units and supposedly depleted mantle sequences. However, it is now known that many ophiolites possess dynamothermal aureoles at their base indicative of high temperature tectonic displacement<sup>35-46</sup> (Fig. 1). Furthermore, the discovery of lherzolites and mafic segregations within ophiolites suggests that the previously accepted bipartite classification of Alpine-type peridotites into primitive high-temperature-high-pressure lherzolite and evolved low-temperature-low-pressure ophiolite types is an oversimplification. Gradation between lherzolite massif and ophiolite end-members appears more credible.

## Mineralogy and textures of the segregations

Most of the mafic segregations are varieties of pyroxenite. The main minerals present in the segregations and host peridotites are shown in Table 1. Clinopyroxene is present in all five mafic samples as augite, salite or diopside: M1, 4 and 5 all contain Ca- and Al-rich salites (fassaite), while M3 comprises diopsides with high jadeite content. Where present, amphiboles are pargasites or kaersutites, plagioclase is anorthitic and garnets pyrope- and almandine-rich. Spinels are olive green varieties of pleonaste. Orthopyroxenes and olivines are Mg-rich. Except for M2, all these mineral compositions indicate high temperatures and pressures of formation: 900–1,400 °C and 17–20 kbar were obtained from mafic segregations in the Zlatibor ophiolite<sup>12,38</sup>; 900–950 °C and 7–10 kbar at the contact between peridotite and metamorphic sole in the White Hills Peridotite<sup>40</sup>,

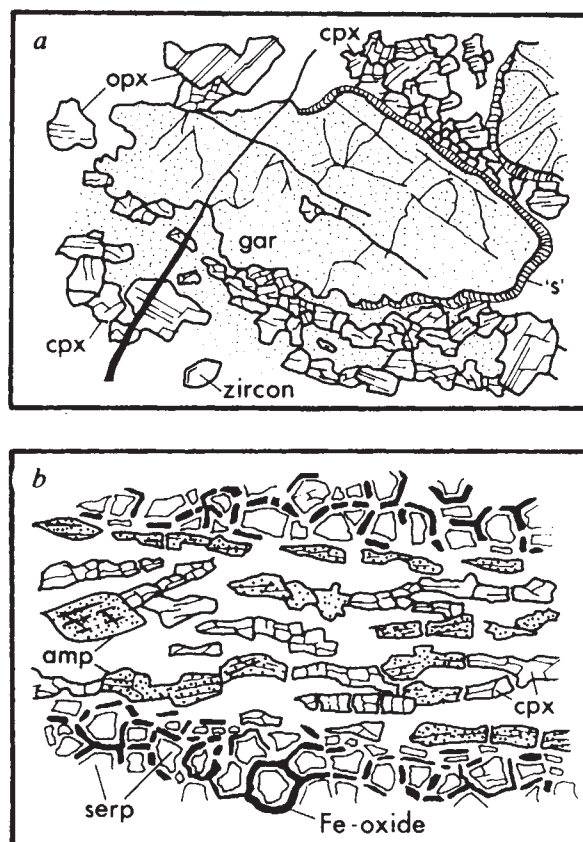


Fig. 2 Mineral textures in: a, garnet pyroxenite mafic segregation in lherzolite from within the Zlatibor ophiolite (box length 10 mm); and b, amphibole-clinopyroxene segregation in serpentinized lherzolite from the Lizard Complex (box length 5 mm). Cpx, clinopyroxene; opx, orthopyroxene; gar, garnet; 's', symplectic reaction rim; amp, amphibole; serp, serpentinized olivine.



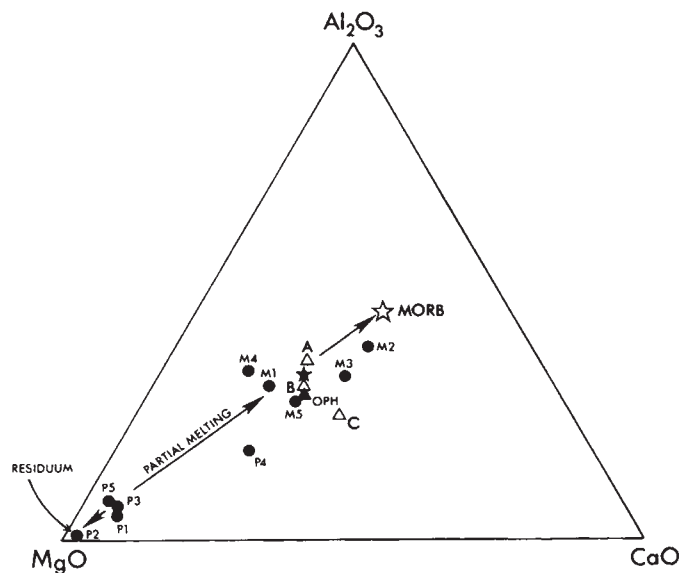


Fig. 3  $\text{Al}_2\text{O}_3$ - $\text{CaO}$ - $\text{MgO}$  plot of the samples in Table 1. Also shown is a mean MORB (average of 94 selected ocean-floor basalt analyses)<sup>49</sup> and parental magma estimated from the mean bulk composition of crustal sequences from four ophiolites (OPH)<sup>52</sup>. The mean position for samples M1-5 is denoted by the black star. The directions in which partial melt and residuum progress from a garnet lherzolite parent are indicated by arrows.

and 700–850 °C and 15–20 kbar from mafic segregations in the Othris and Ballantrae ophiolites<sup>14</sup>. These pressures indicate depths of between 23 and 66 km, all of which are well in excess of the known thicknesses of ophiolites (<15 km), so it is clear that the segregations were neither formed nor re-equilibrated in their present positions in ophiolite mantle sequences. Examples of mineral textures of mafic segregations are shown in Fig. 2. Two of the most dominant fabrics developed in the segregations are granoblastic textures, where there is evidence of brittle deformation (Fig. 2a), and banded textures, where minerals are generally found aligned with metamorphic peridotites (Fig. 2b). These textures are indicative of sub-solidus recrystallization or cataclasis related to post-magmatic events.

## Segregation chemistry

Chemistry and CIPW norms of the mafic segregations and host peridotites from the selected ophiolite mantle sequences are shown in Table 1. All segregations (M1–5) are olivine, pyroxene and plagioclase normative. The low total silica (<45%), the high normative olivine (21–46%) and the presence of normative feldspathoids and corundum indicate undersaturation and an ultrabasic character. The  $\text{MgO}$  contents are particularly high (14–23%) and suggest a picritic affinity for all five samples. They also show similarities with other examples of supposed primitive 'basalts', including those derived experimentally<sup>47</sup>, mafic segregations found in lherzolite massifs<sup>29</sup> and in subcontinental mantle xenoliths<sup>48</sup> (samples A, B and C of Table 1).

The primitive nature of the segregations is also revealed when their compositions are plotted on an ACM diagram (Fig. 3). Both segregations and peridotite hosts lie on or close to the residuum–garnet lherzolite–MORB trend. P1, P3 and P5 approximate Ringwood's pyrolite<sup>50</sup> and the mean composition of 112 oceanic lherzolites<sup>51</sup>. P2 shows a more depleted nature balanced by a more 'evolved' position for M2. P4 appears to be a partial melt product rather than a parental or residual phase. M1–5 plot around the locations of proposed parental magmas to mid-oceanic ridge basalts<sup>47</sup> (A) and ocean crust<sup>52</sup> (OPH). The mean of the five mafic segregations (black star) is also close to these and to sample B of the Lherz massif (see Fig. 3).

The bulk  $\text{CaO}$  contents of M2, M3 and M5 are high, but calcium-rich picrites have been described from megacrysts and glass inclusions in oceanic tholeiites<sup>53</sup> and from certain  $\text{MgO}$ -rich dykes in ophiolites<sup>52</sup>. Elthon<sup>52</sup> argues that an increase in calcium content during fractionation of primary melts may lead to the formation of high  $\text{CaO}$  picritic magmas intermediate to primary melt and oceanic tholeiite. The chemistry, mineral paragenesis and setting of especially the M2 sample<sup>8</sup> concur with this interpretation and with its position in Fig. 3. However, metasomatism may have also affected the segregations: the  $\text{FeO}^*$  contents of M2 and M5 are particularly low, but high in M4 and there is considerable range in alkali contents. Nevertheless, despite the fact that the mafic segregations have probably undergone some chemical modification since their formation and have been selected from different ophiolite localities, all five samples retain qualities characteristic of primitive magmas which is very unlikely to be coincidental. In fact, the average  $\text{MgO}$  and  $\text{Mg}/\text{Mg} + \text{Fe}$  values for M1–5 (18% and 78 respectively) are very similar to those predicted for parental magmas to oceanic tholeiites<sup>52–55</sup>.

## Origins and evolution of the segregations

It is widely accepted that high- $\text{MgO}$  picritic or ultrabasic liquids are the primary magmas which in suitable pressure–temperature conditions undergo fractionation to yield tholeiitic basalts at mid-ocean ridges<sup>52–55</sup>. Yet natural occurrences of picrites are rare, especially from the Phanerozoic period, and this has led some workers to doubt their role in the generation of evolved basalts<sup>56</sup>. However, it has been shown that picritic melts are of high density and would tend to pond at the base of magma reservoirs rather than penetrate through to the surface<sup>57,58</sup>. Because of the fundamental role picritic liquids are considered to have in the generation of basalts, coupled with their relative scarcity at crustal levels, their probable occurrence in the mantle sequence of ophiolites is of particular significance.

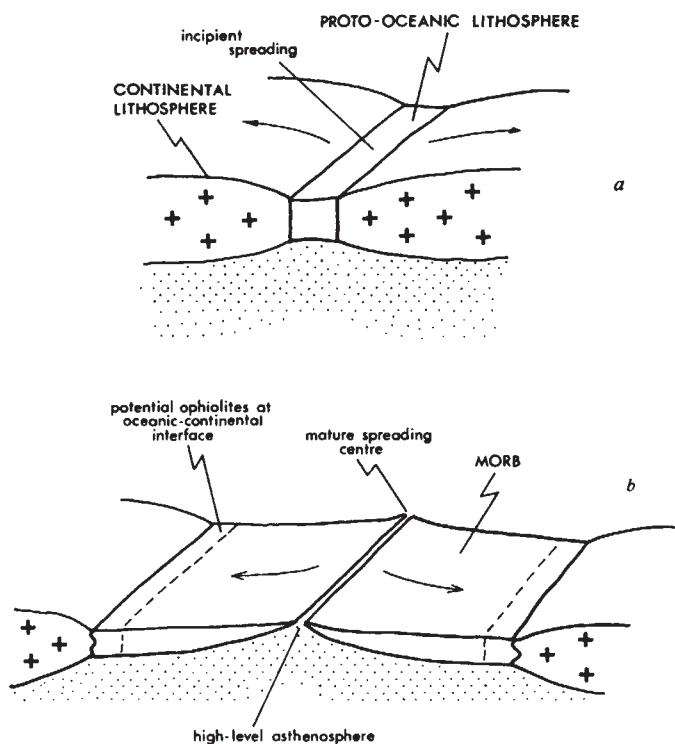


Fig. 4 Simplified model illustrating the inception of an ocean basin. Potential ophiolites possessing components of relatively undepleted mantle, high-temperature–high-pressure mafic segregations and thin crusts are produced at the onset of spreading (a) and hence remain adjacent to continental margins (b) from where they eventually may be emplaced.

For the mafic segregations described here to be preserved at relatively shallow levels (<15 km) and avoid fractionation and re-equilibration they must have undergone ascent within a 'normal' geothermal gradient rather than one found beneath established spreading centres where the isotherms are elevated. Their preservation is even more remarkable when it is considered that sub-solidus, metasomatic and retrogressive modification is likely to have taken place during subsequent ophiolite decoupling and transport. It is not surprising therefore to find that fresh examples of mafic segregations are rare while retrogressed and weathered varieties hosted by heavily serpentinized peridotites are more common.

Preservation of the mafic segregations may also have been aided by low degrees of partial melting at the source area. Below ~5% partial melting it is believed that permeability and interstitial magma flow is prevented so that melts remain as isolated blebs and pockets instead of rising to crustal levels<sup>59</sup>. For the mafic segregations studied here to remain trapped, the peridotite hosts must have undergone rapid ascent so that further fusion was prevented. After and/or during ascent some of the segregations underwent sub-solidus deformation because they are now found as elongate lenses and layers aligned with ultramafic tectonite fabrics. These fabrics were probably formed during asthenospheric upwelling and by plastic flow occurring beneath the spreading centre.

The evolution of the mafic segregations is further complicated by the effects of ophiolite decoupling and transport. Many ophiolite mantle sequences show evidence of intense deformation related to thrusting within 1–2 km of their bases. This basal section is frequently mylonitized and may acquire a new foliation which overprints the earlier tectonite fabric<sup>60</sup>. Lherzolites containing mafic segregations are particularly common at the very base of ophiolites and as a result of this basal deformation are often found severely deformed and fragmented. Recognition of this disruption may help to resolve an outstanding problem regarding metamorphic soles which commonly form along the base of ophiolites<sup>35,36</sup> (Fig. 1). Many workers have been perplexed by the apparent pressure as well as temperature inversion shown by dynamothermal soles as revealed by their mineral parageneses<sup>14–16,38,40,43</sup>. It is more probable that the high pressure components of the soles are in fact mafic segregations which have been tectonically juxtaposed with the main body of the later formed metamorphic sequence. The common occurrence of lherzolites with mafic segregations at the base of ophiolite mantle sequences would make tectonic interleaving with a dynamothermal aureole a likelihood during overthrusting. In this respect, metamorphic soles may comprise two dis-

tinct components: a conventional schistose aureole formed during overthrusting and high-temperature-high-pressure mafic segregations formed before thrusting which have become inter-banded with the upper part of the aureole.

## Conclusions

The occurrence of mafic segregations as a minor component of ophiolite mantle sequences is of interest because their compositions closely resemble that postulated for parental magma to oceanic tholeiite. The high-temperature-high-pressure mineralogy of many of the segregations also concurs with the probable depths of generation of hypersthene- or nepheline-normative picritic magmas in the upper mantle. The lower-temperature-lower-pressure parageneses with more evolved bulk compositions shown by other segregations imply that re-equilibration and fractionation have affected some examples. Preservation of high pressure segregations requires rapid uplift to avoid re-equilibration and significant fractionation and to facilitate relatively high level inclusion in ophiolite complexes. Such rates of ascent would not normally be associated with adiabatic decompression required for volume production of MORB at oceanic spreading centres. Rather they imply tectonic or unusually rapid diapiric uprise more akin to incipient rifting and the start of spreading or to intermittent spreading. These conditions are unlikely to prevail beneath a 'mature' spreading centre where thermal gradients are high enough to permit partial melting and fractionation at shallow depths. Instead, it is more likely that partial fusion would be restricted to deeper levels in the upper mantle with the production of more primitive melts. Corroborative evidence for incomplete fusion is afforded by the apparent reduced thickness above the Moho shown by many ophiolites when compared with mature oceanic crust (about 4 km compared with 7 km respectively<sup>61,62</sup>). I conclude that ophiolites possessing relatively undepleted mantle, high-temperature-high-pressure mafic segregations and thin crusts were produced during the early stages in the evolution of ocean basins and do not represent fragments of normal oceanic crust and upper mantle (Fig. 4). Such ophiolites are likely to have been formed at the very edge of ocean basins adjacent to continental margins in a prime position for their eventual tectonic emplacement.

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# The primary structure and genetic organization of the bovine papillomavirus type 1 genome

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*The complete nucleotide sequence of the double-stranded circular DNA of bovine papillomavirus type 1 (BPV-1) was determined. Analysis of this sequence in conjunction with known transcriptional data for the virus provides a basis for determining the organization of the papillomavirus genome. All the major open reading frames are located on the same DNA strand. The region transcribed in BPV-transformed cells contains open frames in all three translational frames whereas the region transcribed in productively infected bovine fibropapillomas is characterized by two large open reading frames partitioned by a single translational stop codon. The localization of sequences diagnostic of promoters and polyadenylation sites suggests that splicing is involved in the biosynthesis of the viral mRNAs. A sequence comparison indicates the genomic organization of the bovine papillomavirus and that of the members of the simian virus 40-polyomavirus subgroup to be distinct, suggesting that these two groups of viruses are evolutionarily unrelated.*

STUDIES on the papillomaviruses date back to 1933 when the Shope papillomavirus was first described as the causative agent of cutaneous papillomas in cottontail rabbits<sup>1</sup>. The papillomaviruses are widespread in nature and are responsible for a variety of pathological conditions in humans. To date, however, none of the papillomaviruses has been successfully propagated in the laboratory and as a consequence our knowledge of the biology and genetic organization of this group of viruses is limited. In general, papillomaviruses are highly host specific and replicate only in the terminally differentiated epidermal cells of the papillomas (warts) which they cause. There is a subgroup of papillomaviruses, consisting of the bovine papillomaviruses types 1 and 2 (BPV-1 and BPV-2), the deer fibroma virus and the ovine papillomavirus, which are tumorigenic when inoculated into hamsters<sup>2-4</sup>.

BPV-1 is the most extensively studied of the transforming papillomaviruses. It causes cutaneous fibropapillomas in cattle<sup>5</sup> and a naturally occurring fibroblastic tumour, equine sarcoid, in horses<sup>6</sup>. In the laboratory, it is tumorigenic in pika<sup>7</sup>, domestic rabbits<sup>8</sup> and hamsters. The virus is capable of inducing transformation in mouse<sup>9-12</sup>, hamster<sup>13</sup> and bovine conjunctive<sup>14</sup> cells, and the cloned viral DNA can transform susceptible mouse cells<sup>15,16</sup>.

The structure of BPV-1 is similar to that of other papillomaviruses. It is a small icosahedral DNA virus with 72 capsomers whose genome consists of a double-stranded, circular DNA molecule of molecular weight (MW)  $5.0 \times 10^6$  (ref. 17). The virions contain a major capsid protein of MW 53,000 (ref. 18), possibly some minor capsid proteins and the host histones H2A, H2B, H3 and H4. The region transcribed in BPV-1-transformed cells (and by analogy with other DNA tumour viruses such as simian virus 40(SV40) and polyoma, the region transcribed in epidermal cells before the onset of vegetative viral DNA synthesis) is localized to a single strand and extends over 50% of that strand<sup>19</sup>. Additional RNA transcripts not found in transformed cells are present in the epidermal cells of a bovine fibropapilloma. They originate from the remaining portion of the same strand and encode the major capsid protein (L. Engel, C. A. Heilman and P.M.H., in preparation). To date, nothing is known of the virus proteins expressed in either BPV-1-transformed cells, the dermal fibroblasts of a fibropapilloma, or in the proliferating epidermal cells of the basal layer or stratum spinosum of a fibropapilloma.

Despite the lack of a tissue culture system for the *in vitro* propagation of the papillomaviruses, which to date has precluded detailed genetic and molecular study of these viruses, a large amount of taxonomic information has been accumulated

on the human and bovine papillomaviruses. The purified virus has been used to make specific antisera and the viral DNA has been analysed to yield characteristic restriction endonuclease cleavage patterns and to provide a template for the purification of virus-specific RNA or DNA probes. More recently, using recombinant DNA technology, complete papillomavirus genomes have been cloned directly into *Escherichia coli* plasmid vectors for the generation and standardization of specific papillomavirus molecular reagents<sup>16,20,21</sup>. To further our understanding of the functional organization of the BPV-1 genome, we have now determined, and present here, the complete nucleotide sequence of the viral DNA.

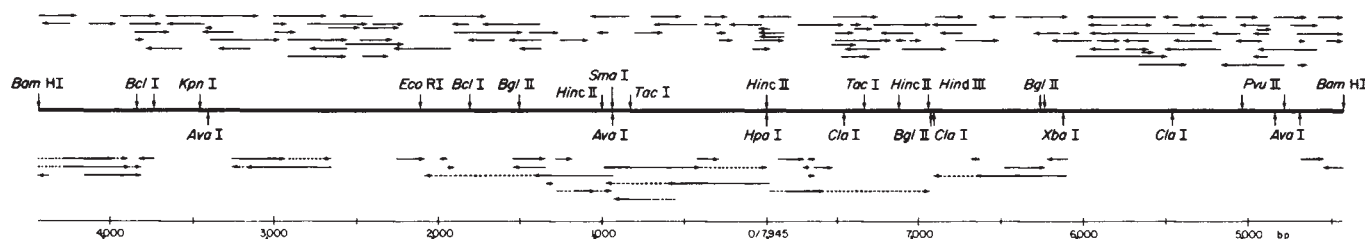
## Sequencing strategy

About 95% of the sequence was obtained by a shotgun approach<sup>22,23</sup> using computer-assisted reassembly from data generated by sequencing with chain terminators<sup>24</sup> single-stranded recombinant phage M13mp7 (ref. 25) DNA templates which contained random overlapping segments from both strands of BPV-1 (isolate 307) DNA. Random segments were generated by partial DNase I digestion in the presence of  $Mn^{2+}$  (ref. 26) from recombinant pBR322 DNA carrying the complete BPV-1 DNA integrated at its single *Bam*HI site. Suitable fragments were isolated by gel electrophoresis and cloned into phage M13mp7 RF-DNA<sup>25</sup>. Colourless plaques containing recombinant phage were grown and aliquots of the phage DNAs spotted on two nitrocellulose filters and probed with radioactively labelled BPV-1 and pBR322 DNA to select templates for sequencing. The shotgun approach yielded a total of 22,500 base pairs (bp) of sequence information which covered ~95% of the genome.

To complete the sequence defined fragments which spanned the remaining gaps were subcloned and sequenced using the recently developed phage vectors M13mp8 and mp9 (ref. 27). These vectors expedite selective sequencing in an elegant manner by accepting restriction endonuclease-generated DNA fragments with a wide variety of heterologous termini. The regions of BPV-1 DNA selected for sequencing by this approach covered a total of 4,800 bp. Figure 1 shows the origin and strandedness of all the sequenced fragments of BPV-1 DNA relative to a linearized map of the viral genome. The entire nucleotide sequence (7,945 bp) of BPV-1 DNA is shown in Fig. 2.

The BPV-1 genome has been opened at the single *Hpa*I site (0/1.0 map units, previously 0.88 map units) and numbering proceeds in the 5' to 3' direction of transcription, initiating with





**Fig. 1** Distribution and strandedness of sequenced DNA templates relative to a map of the BPV-1 genome. BPV-1 DNA is shown as a solid black line and is linearized at the unique *Bam*HI site used for generating pBPV-1 DNA. The recognition sites of several restriction endonucleases are shown as landmarks. The arrows above the map show the length and 5' to 3' direction of single-stranded portions of the viral DNA cloned into phage M13mp7 DNA. To generate these templates, plasmid pBPV-1 DNA was digested at room temperature with DNase I (ref. 26) in 0.5 ml 50 mM Tris-HCl, pH 7.5, 30 mM NaCl, 1 mM MnCl<sub>2</sub>, containing 500 µg DNA, 50 ng bovine serum albumin and 20 ng pancreatic DNase I (Sigma). Aliquots of 160 µl were withdrawn after 16, 20 and 24 min, digestion was stopped by the addition of EDTA to 5 mM and aliquots were combined, extracted with phenol and the DNA precipitated with ethanol. To maximize blunt ends, DNA was incubated with *Escherichia coli* DNA polymerase I (10 U of large fragment; Boehringer) for 30 min at 15 °C in 300 µl of 20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 6 mM MgCl<sub>2</sub>, 50 mM NaCl, 1 mM dithiothreitol and 50 mM each of the four deoxyribonucleotide triphosphates (dCTP was set to a specific activity of 1,000 c.p.m. pmol<sup>-1</sup> using [ $\alpha$ -<sup>32</sup>P]dCTP; Amersham). DNA was recovered by phenol extraction and ethanol precipitation and size-separated on a 10% polyacrylamide gel. DNA 300–1,000 bp long was recovered by electroelution followed by ethanol-precipitation from the eluate. Recovered DNA (~10% of the DNA loaded on the gel) was ligated to endo *Hinc*II-cleaved M13mp7 RF-DNA (100 ng) and the ligation mixture was used to transform competent *E. coli* JM 103 cells. Transformation and plating of transformed cells were performed as described in ref. 25. Template DNAs prepared<sup>25</sup> from 164 recombinant (colourless) plaques were dissolved in water in a final volume of 15 µl. Aliquots of 1 µl added to 1 µl 0.5 M NaOH, 1 M NaCl were spotted onto each of two nitrocellulose filters and filters were processed for hybridization<sup>39</sup> with radiolabelled probes to pBR322 DNA and BPV-1 DNA<sup>23</sup>. Filter-spotted DNAs which gave a signal with the BPV-1 DNA probe and some of those which hybridized with both probes were selected for sequencing. The arrows below the genomic map indicate the templates whose sequences filled gaps in, and confirmed, data obtained from the shotgun approach. These templates were generated by cloning defined restriction fragments with heterologous and homologous termini into phage vectors M13mp8 and mp9 RF-DNA<sup>27</sup>. Typically, the desired fragment was obtained after polyacrylamide gel electrophoretic separation of 1 µg of pBPV-1 DNA cleaved with the appropriate restriction enzyme(s) and isolation from the gel. Fragments (~50 ng) were ligated with T4 DNA ligase to singly or doubly cleaved M13mp8 or mp9 RF-DNA (20 ng). The small arrows represent a selection of sequenced endo *Alu*I-generated fragments obtained by cloning gel-isolated *Alu*I-cleaved BPV-1 DNA into the *Sma*I site of phage M13mp8 RF-DNA.

the first of the six nucleotides of the *Hpa*I site. In previous studies on BPV-1 RNA transcripts in transformed cells<sup>19</sup>, map positions were based on the unique *Hind*III site and the non-coding strand was numbered.

## Open reading frames

The analysis of the nucleotide sequence for each of the possible translational reading frames revealed that all the significant open reading frames are located on one DNA strand. Approximately 90% of this strand contains long open reading frames. The only significant region which has no open frames lies between 0.90 and 0.01 map units (from base ~7,150 to ~80). Figure 3 shows, for all the potential reading frames, the translational maps of both strands of the BPV-1 genome with the open reading frames greater than ~500 bases long. There are several small open reading frames of up to 450–500 bases located on the opposite strand; however there is no evidence that transcripts complementary to that strand are made in transformed or productively infected cells (ref. 19; L. Engel, C. A. Heilman and P.M.H., unpublished).

## The transforming region

Using the quantitative assay described by Dvoretzky *et al.*<sup>11</sup> with the cloned BPV-1 DNAs, it has been possible to localize the region necessary for BPV-mediated transformation to a specific segment making up 69% of the genome<sup>15,16</sup>. This region is bounded by the *Hind*III (base 6,958) and *Bam*HI (base 4,450) sites. Attempts to reduce the size of this region by cleaving within this segment at the *Hpa*I (base 1), *Kpn*I site (base 3,455), or the *Eco*RI (base 2,113) sites result in a loss of transformation activity.

Recent studies mapping the transcripts present in BPV-1-transformed mouse cells have provided further information concerning the genetic organization of the transforming region. Five distinct transcripts were detected and mapped in BPV-1-transformed cells (see Fig. 4). The 3' end of each of these transcripts maps at approximately 0.53 map units and the 5' ends of the bodies of distinct transcripts map at approximately 0.03, 0.09, 0.34, 0.39 and 0.41 map units<sup>19</sup>. In this study, no splicing was demonstrated, but the experimental conditions were such that a small 5'-leader sequence would not have been detected.

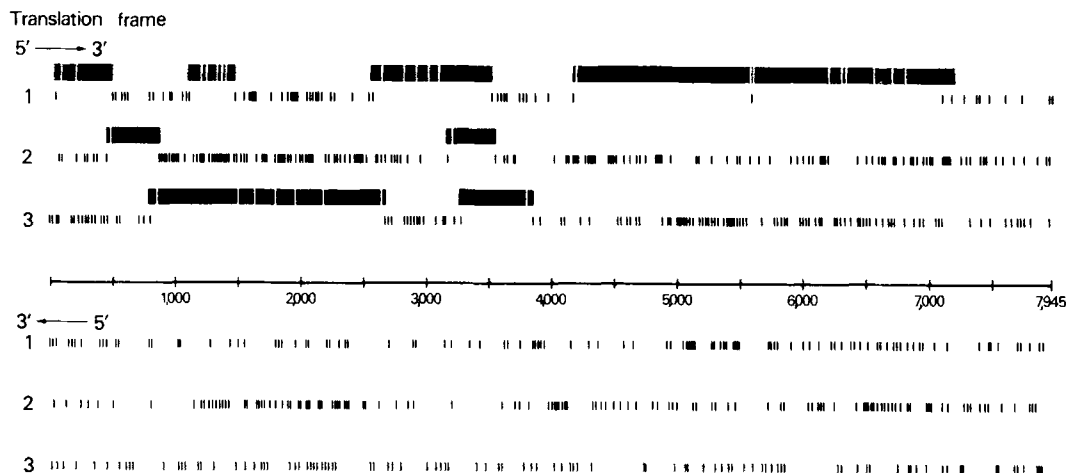
The sequence analysis of this region reveals several open translational frames in the coding strand involving each of the three possible frames (Fig. 3). Within this region there are two TATAAA sequences (bases 7,108 and 58), located just upstream from the 5' end of the body of the largest RNA transcript identified in the transformed cells (Fig. 4). The TATAAA sequence diagnostic of a eukaryotic promoter is located around base -30 of the cap site of viral and cellular mRNAs and is thought to be involved in the proper positioning of the 5' ends of these mRNAs<sup>28</sup>. Which, if either, of the two potential promoter elements is involved in the biogenesis of the viral transcripts in the transformed cells is under investigation. There is a polyadenylation recognition sequence, AATAAA, at base 4,179 (0.53 map units) which corresponds well with the 3' ends of the RNA transcripts identified in BPV-transformed cells. Thus, the sequence analysis is consistent with the transforming region containing a single transcriptional unit consisting of promoters at either 0.90 or 0.01 map units, coding sequences mapping from 0.03 to 0.53 map units and a polyadenylation site at 0.53 map units. The different transcripts detected in transformed cells could therefore be

**Table 1** Segments of dyad symmetry located in the non-coding region of the BPV-1 genome

| Sequence       | Location    | Internal segment size |
|----------------|-------------|-----------------------|
| GTGCA TTGAATTA | 7,240–7,253 | 88                    |
| CACGTCACTGAAT  | 7,152–7,139 |                       |
| AGAACTAAGC     | 7,331–7,341 | 222                   |
| TCTTTGAATCG    | 7,108–7,098 |                       |
| GCTTTT TTTT    | 7,435–7,444 | 336                   |
| CGAAATAAAA     | 7,098–7,089 |                       |
| TTTTTGGAGGATTA | 7,568–7,581 | 486                   |
| AAAAACGTCGAAT  | 7,081–7,098 |                       |
| ATGCAGCATTA    | 7,872–7,882 | 265                   |
| TACGTCCTAAT    | 7,606–7,596 |                       |
| GGGAAAAATA     | 37–47       | 50                    |
| CCCTTTTAT      | 7,931–7,921 |                       |

**Fig. 2**

**Fig. 2** The nucleotide sequence of BPV-1 DNA. The entire sequence (7,945 bases) was obtained by computer-assisted assembly from partial sequence data as described in the text. All DNA templates were sequenced by the dideoxynucleotide chain termination method<sup>24</sup> using a 15 nucleotide long synthetic primer<sup>25</sup>. Sequencing reactions (5 µl, 300 ng template DNA) were terminated by the addition of 10 µl of 98% deionized formamide, 10 mM EDTA, 0.2% bromophenol blue and 0.2% xylene cyanol. Terminated reaction mixtures were heated for 3 min at 100 °C and 1-µl aliquots were electrophoresed on 40 cm long 5% polyacrylamide 8M urea 'thin' gels<sup>43</sup> for 2–6 h at 30 mA and 18 kV. Gels were then transferred onto Whatman 3MM paper, vacuum-dried and exposed to X-ray film for an average of 12 h. The presumed coding strand of BPV-1 DNA is shown, starting with the nucleotides which constitute the unique *Hpa*I site. The locations of cleavage sites for several restriction endonucleases are indicated.



**Fig. 3** Open reading frames in BPV-1 DNA. The entire length (7,945 nucleotides) of BPV-1 DNA opened at the unique *Hpa*I site is depicted in the middle of the figure. The rows of small vertical lines shown above and below the genomic map indicate the positions of the translational stop codons TAA, TAG and TGA found in the translational reading frame of the two DNA strands. Horizontal black bars indicate regions of 500 nucleotides or more which lack a termination codon. White lines superimposed on these bars indicate the positions of the translational initiation codon ATG. Note that the largest bar contains one stop codon.

generated by differential splicing using a common 5'-end leader sequence. There is a potential cap site (ACA) located 31 bases downstream from the TATAAA sequence at position 58. A potential splice donor sequence (AG<sup>1</sup>GTGCAT) is located at base 183, which if used in the biogenesis of the BPV-1-transformed mRNAs, could result in a leader sequence of approximately 100 bases which might be spliced to each of the five bodies mapped in the transforming region. There is also an ACA cap sequence 25 bases downstream from the potential TATAAA promoter at base 7,108. Interestingly, a potential splice donor sequence (AG<sup>1</sup>GTAAGT) occurs at base 7,141, which would result in a leader sequence of only six bases that could be spliced to the bodies of the viral mRNA species in transformed cells. Potential splice acceptor sites (that is CAG or TAG preceded by sequences rich in pyrimidines and devoid of an AG dinucleotide) are located in the regions of the 5' ends of each of the bodies of the RNA transcripts from the transforming region of the genome. The sequence data are therefore consistent with the experimentally unproved prediction that the viral RNAs expressed in transformed cells are derived from a single transcriptional unit and are generated by differential splicing.

Interestingly, the end of the transforming region of BPV-1 was shown to contain a transcriptional activator ('enhancer') element. Lusky and Botchan (unpublished) found that a DNA segment which spans the polyadenylation site at 0.53 map units can substitute for the 72 bp tandem repeats of SV40 DNA. The relevance of this enhancer for the expression of BPV genes remains to be elucidated.

### Capsid protein-encoding region

The 32% of the BPV-1 genome mapping between the *Bam*HI and *Hind*III sites (bases 4,450–6950) is not expressed in BPV-1-transformed cells<sup>19</sup> and is not required for BPV-1 DNA-mediated transformation<sup>15</sup>. This region is spanned by a single open reading frame which extends from nucleotides 4,171 to 7,095, with a termination codon located in frame at base, 5,593 (see Fig. 3). Analysis of the viral RNA transcripts present in bovine fibropapillomas which contain epidermal cells in which productive replication of BPV-1 virus is occurring reveals species of approximately 1,800 and 4,000 bases which are transcribed from this region (L. Engel, C. A. Heilman and P.M.H., unpublished). This same region has been shown to encode the major capsid protein (MW 53,000) of BPV-1 by filter selection of these mRNA species and translation *in vitro* (C. A. Heilman *et al.*, unpublished). The 3' ends of these

transcripts map to 0.91 map units, where two polyadenylation sites (at nucleotides 7,083 and 7,146) are found. The precise structure and 5' ends of the productive mRNAs are not yet known, thus the amino acid sequence of the 53,000 MW major capsid protein, which would be encoded by ~1,300 bases of the open 3,000 bases in this region, cannot yet be predicted (see Fig. 3). Certain minor capsid proteins such as VP2 and/or VP3 may also be encoded in this region, as may non-structural proteins which are not expressed in BPV-transformed cells.

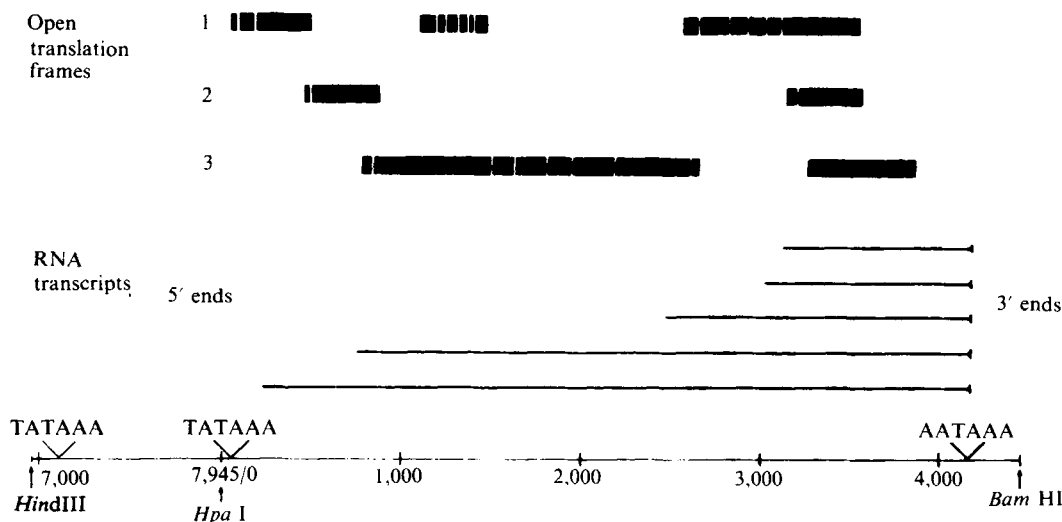
Given that papillomas in cattle represent an economic problem of some consequence, the identification of genes encoding potential capsid proteins revealed by the DNA sequence may permit the development of a vaccine against bovine papillomas through the design of either synthetic peptides<sup>29,30</sup> or production of the capsid protein(s) in a heterologous host<sup>31</sup>.

### Noncoding region

There is a region of approximately 1,000 bases of the BPV-1 genome between nucleotides 7,146 and 49 in which there are no significant open reading frames. This region is bound on one side by the 3' ends of the productive messenger RNAs (positioned by the polyadenylation sites at base 7,146) and on the other side by an open reading frame in the transforming segment beginning at base 49. By analogy with other well studied virus systems, this noncoding region probably harbours important virus transcription regulatory sequences as well as the sequences comprising the origin of DNA replication.

Within this 1,000 base region no significant repeated sequence units are found analogous to the 72 and 68 bp tandem repeated sequences present in the noncoding regions of SV40<sup>32,33</sup> and BK<sup>34,35</sup> genomes respectively. Yet certain sequence features in the noncoding region of the BPV genome are shared with those origins, suggesting that this region may contain the origin of vegetative DNA replication which functions in epidermal cells. Common sequence features of the papovavirus origins of DNA replication include an A+T-rich region, palindromic sequences and inverted repeated sequences. The noncoding region of the BPV-1 genome contains several A+T-rich regions including one 21 base run (7,077–7,097), a 12 base run (7,879–7,890) and three 10 base runs (7,154–7,163 7,395–7,404 and 7,683–7,692) which contain only A–T residues. Several near-perfect palindromes are located in this region, including CTGAATTTTAATTC (bases 7,392–7,405), TTTGAGGATT (bases 7,570–7,580) and ATGTCTGTA (bases 7,612–7,620). In addition, several areas of dyad symmetries with non-homologous loops are present,





**Fig. 4** The composite structure of the BPV-1 transforming segment. A map of the BPV-1 transforming segment (*Hind*III to *Bam*HI subgenomic fragment) is shown in linear form on the bottom line. The bodies of the polyadenylated RNA transcripts found in BPV-1-transformed mouse cells<sup>19</sup> are indicated. The AATAAA sequence (bases 4,170–4,184), which presumably is the polyadenylation recognition site for this set of RNAs, is positioned on the map. Potential TATAAA promoter elements located at bases 7,108–7,113 and 58–63 are indicated. The horizontal bars above the RNA transcripts represent potential coding regions for BPV-1 proteins in each of the three reading frames (see also Fig. 3).

including AAAGCGAAAGCGACACGCTTT (bases 7,419–7,439). Six segments of dyad symmetry are located in this region with various-sized internal non-homologous loops (see Table 1). Although these sequence features are intriguing, functional data are required to support the contention that the origin of replication is, in fact, in this region.

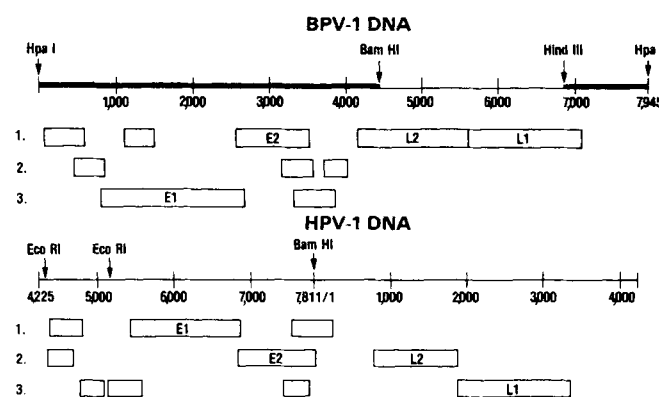
## Conclusions

The absence of a tissue culture system to propagate the papillomaviruses has precluded their analysis by standard virological procedures. Consequently, although these viruses have been studied since 1933, virtually nothing is known of the genetic organization of the papillomavirus DNAs or of the elements controlling viral gene expression. A number of laboratories have successfully cloned the genomes of various papillomaviruses<sup>16,20,21</sup>, and the recent advances in DNA sequencing technology have made the rapid sequencing of an 8 kilobase genome feasible. The entire sequence of the 7,945 base DNA of BPV-1 presented here, in conjunction with the transcriptional data known for this virus<sup>19</sup>, provide a basis for deducing the genetic organization of the papillomavirus genome.

Only one strand of the genome contains significant open reading frames and transcripts from only this strand can be detected in transformed cells and in productively infected fibropapillomas of cattle. This contrasts with the SV40–polyoma subgroup of viruses and the adenoviruses, where sequences from both DNA strands are transcribed in productively infected cells<sup>17</sup>.

The complete genome of a human papillomavirus (HPV-1a) has also recently been sequenced and found to contain 7,811 bp (ref. 36). As is the case with BPV-1 all the genetic information appears to be encoded on a single strand of the genome. These two genomes have been shown previously to be genetically related and up to ~25% nucleotide sequence homology has been detected and mapped within each genome<sup>37</sup>. Using these homologous regions it is possible to align the two genomes (Fig. 5). The first base of the BPV-1 genome corresponds approximately to base 4,225 in the HPV-1a genome. The open reading frames predicted from each of the sequences in each of the three possible translation frames for the coding strands are depicted below the linearized and aligned genomes. Previous homology studies<sup>37,38</sup> and a comparative analysis of the primary sequence data define two regions which are highly conserved between these two genomes and which map to the

sequences corresponding to the open reading frames designated L1 and E1. We have used the nomenclature proposed by Dano *et al.*<sup>36</sup> and designated the two large reading frames spanning the region not essential for transformation as L1 and L2, and the two largest open reading frames in the transforming region of BPV-1 as E1 and E2. A comparison of the genetic organization of these genomes reveals marked similarities. The size and position of each of the major open reading frames are quite similar. In BPV-1, L2 and L1 are located in the same open reading frame and are partitioned by a single stop codon, and in HPV-1, the 3' end of the L2 reading frame overlaps the 5' end of L1 by five bases and is located in a different reading frame. In the regions corresponding to the transforming segment of BPV-1, there is a noncoding segment ~1,000 base long, 5' to a series of small open reading frames preceding the



**Fig. 5** Alignment of BPV-1 and HPV-1A genomes. The linearized genomes were aligned (HPV-1a base, 4,225 corresponding to BPV-1 base 1) using regions of conserved nucleotides between the DNAs. Open reading frames for each of the three potential translation frames are depicted beneath the genomic maps. The 69% transforming segment of the BPV-1 genome is indicated by a heavy bar. The two large open reading frames spanning the region not essential for transformation and located in the corresponding region in HPV-1a are labelled L1 and L2 in agreement with the proposed nomenclature<sup>36</sup>. Similarly, the two largest open reading frames in the transforming segment of the BPV-1 genome and the corresponding open reading frames in HPV-1a are designated E1 and E2.

large E1 reading frame which is highly conserved. The 5' end of the E2 reading frame overlaps the 3' end of the E1 reading frame in each genome and there is some conservation of amino acids near the amino terminus in the putative proteins encoded by the E2 reading frames. A more thorough analysis of these two genomes is currently the subject of a collaborative study.

The papillomaviruses (subgroup A) and the SV40-polyoma virus (subgroup B or miopapovavirus genus) of viruses together form the papovavirus family of viruses<sup>39</sup>. The term papovavirus derived from the first two letters of each of the known members at the time it was proposed: papillomavirus, polyomavirus and vacuolating virus (SV40)<sup>40</sup>. Although these two groups of viruses are morphologically similar, the papillomaviruses are larger than the SV40-polyomaviruses and comparative studies have suggested that these two virus groups are unrelated. In non-stringent hybridization conditions capable of detecting conserved genomic regions with as much as 33% base mismatch, no homology could be detected between papillomavirus DNAs and those of SV40 or BK DNA<sup>37</sup>. Highly conserved capsid antigenic determinants detectable in all known members of the SV40-polyomavirus group are not present in papillomaviruses; and conversely, a highly conserved cross-reactive antigenic determinant found in all known papillomaviruses is not present in the SV40-polyomavirus group<sup>41</sup>. The sequence analysis presented here shows convincingly, however, that the SV40-polyomavirus group and the papillomaviruses are evolutionarily distinct. The genetic organization of the papillomaviruses is different from that of the polyomaviruses and there is no sequence homology between the coding and non-coding regions of SV40 and BPV-1 DNAs.

There are only limited data available concerning BPV-1 transcription, but it is clear that cellular controls regulate BPV-1 gene expression. In mouse cells, only a portion of the viral genome is expressed. No capsid proteins are synthesized, nor are transcripts complementary to the sequences which encode these proteins observed. In a bovine fibropapilloma, capsid proteins are not produced in the mesenchymal fibroblastic component or in the epidermal cells of the basal layer or the lower stratum spinosum. Only in the terminally differentiated epidermal cells of the granular layer are the capsid proteins detectable. It seems likely, therefore, that the regulation of viral gene expression in the fibropapilloma is at the level of transcription. Two additional transcripts are present in bovine fibropapillomas which are not present in transformed cells. These transcripts are complementary to the sequences which encode the BPV-1 capsid proteins and must therefore be expressed in the terminally differentiated epidermal cells in which capsid protein production occurs. Thus, expression of viral productive functions, such as capsid protein synthesis, are linked to the state of epidermal cell differentiation.

There are three TATAAA sequences (bases 58, 5,089 and 7,108) and two TATATA sequences (bases 4,072 and 6,859) in the coding strand of the BPV-1 genome. These sequences have been shown to be important in localizing the 5' ends of transcripts *in vivo* and *in vitro* when part of a eukaryotic promoter. The TATAAA sequence located at base 58 maps near the 5' end of the largest RNA transcript detected in BPV-1-transformed cells. It seems possible that this TATAAA sequence and the surrounding region define the viral promoter active in transformed cells.

From the DNA sequence it is not clear what transcriptional promoter(s) function in the biosynthesis of the transcripts for the productive messages for the capsid proteins. These mRNAs are complementary to sequences located between bases 4,450 and 7,945. There is a TATATA sequence, which might function as part of the promoter located, at bases 4,072–4,077, just 5' to a large open reading frame starting approximately at base 4,150. Alternatively, the promoter active in the transformed cells may function for the biosynthesis of the productive transcripts and the control of the gene expression may be a result of differential splicing. There are additional TATAAA sites on this strand at bases 5,089–5,094 and 7,108–7,113, and a TATATA sequence at bases 6,859–6,864, any of which may be part of the productive promoter. The definition of the productive transcriptional unit must await precise mapping of the productive transcripts and their 5' ends.

Due to the ability of molecularly cloned BPV-1 DNA to transform mouse cells<sup>15,16</sup>, the possibility of exploiting BPV-1 DNA as a novel eukaryotic cloning and expression vehicle was suggested. The utility of this approach has been demonstrated using the rat preproinsulin gene. It has recently been reported that cells transfected with plasmid DNA containing both BPV-1 and rat preproinsulin sequences can be transformed, and that such transformants harbour the recombinant DNA in an episomal state, directing the expression of proinsulin<sup>42</sup>. The availability of the complete nucleotide sequence should greatly facilitate efforts to enhance the utility of BPV-1 DNA as a cloning/expression vehicle, and will provide the framework required to complete our understanding of the molecular biology of the BPV life cycle.

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## LETTERS TO NATURE

## Bismuth abundance anomaly in a Hg–Mn star

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Strong absorption lines due to Bi II have been found in the Hg–Mn star HR7775 (HD193452) in high-resolution spectra obtained with the IUE. HR7775 was first recognized as a chemically peculiar star by Preston in 1972. Detailed examination of the optical spectrum at high resolution<sup>1</sup> showed that it is one of the most extreme stars of the 'cool' Hg–Mn group, with strong enhancements of Hg, Pt, Sr, Y, and Ga; the last of these is confirmed<sup>2</sup> by the very strong Ga II resonance line at 1,414 Å. Four-colour Strömgren photometry of HR7775 (ref. 3), interpreted with the aid of the model atmosphere calibrations by Relyea and Kurucz<sup>4</sup>, gives  $T_{\text{eff}} = 10,800$  K,  $\log g = 4.2$ , while the H $\beta$  index gives  $\log g = 4.0$  according to the calibration of Schmidt<sup>5</sup>. We have adopted a fully blanketed model atmosphere<sup>6</sup> with solar composition,  $T_{\text{eff}} = 11,000$  K,  $\log g = 4.0$ . Uncertainties in the exact composition and parameters have a very small effect on our results when compared with uncertainties due to oscillator strengths, identification of blending lines, and correct placement of the unblanketed continuum. The overabundance of Bi obtained from spectrum synthesis calculations is  $\sim 10^6$ . Close examination of the spectra of several other Hg–Mn stars shows that Bi II is not detected in any other star in this group.

The IUE spectra were processed at the University College London Starlink node using the standard analysis programs STAK and TRAK. In Fig. 1 we compare the region of the Bi II resonance line at 1,436.83 Å in SWP images of three stars: HR7775,  $\iota$  CrB (Hg–Mn) and  $\nu$  Cap (normal B9.5 V). The spectra of these three stars match well except at the wavelength of Bi II, and at a strong adjacent feature which is due to an antimony line (Sb II 1,436.49 Å) in the two chemically peculiar stars. The existence of an antimony anomaly will be discussed elsewhere.

In addition to the resonance line we have found 30 other strong UV features, present in HR7775 but in most cases absent in  $\iota$  CrB and the other stars studied. These 31 lines correspond with all of the strong lines listed by Crawford and McLay<sup>7</sup> in the interval 1,240–1,980 Å. Three of the stellar features are blends with very strong lines in all stars studied, and cannot be analysed further. In view of the differential nature of the comparison between HR7775 and the other stars, some of which are very similar to it in chemical composition (in particular for iron-peak elements), the identification of Bi II must be regarded as well established.

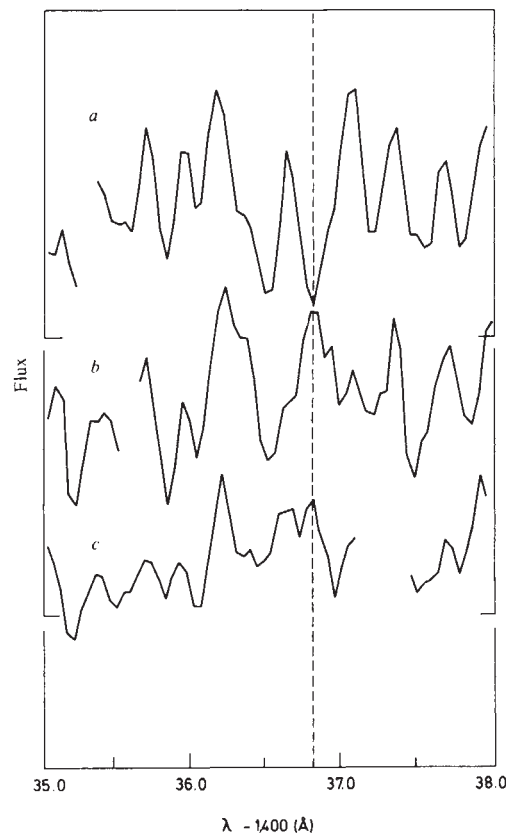
An analysis has been performed by calculating a synthetic spectrum of blended absorption lines with a computer program developed by J. Ross at UCLA and modified by us specifically

for analysing IUE spectra. Examples of synthesis results are shown in Fig. 2. We assume local thermodynamic equilibrium and a homogeneous distribution of all elements with depth. The Voigt profiles of strong lines are calculated assuming that radiation damping is the dominant mechanism; the classical damping constant is adopted for weak lines, while for strong lines the values have been calculated from the lifetimes of the levels involved, when known. The line opacities at each wavelength and depth are summed and added to the continuous opacity, and the emergent flux distribution is calculated at intervals of  $\Delta\lambda = 0.01$  Å. The emergent flux is then convolved with a gaussian profile representing the instrumental broadening of the IUE spectrograph. The  $e^{-1}$  full-width of the instrumental profile was obtained from our study of spectra of stars with  $v \sin i \leq 2$  km s<sup>-1</sup>, and found to be ( $\lambda$  in Å):

$$0.15 + [0.0077 (\lambda - 1,350)/100] \text{ Å}$$

for SWP spectra, and 0.20 Å for LWR spectra. In the case of bismuth we have not found any tabulated partition functions; these were calculated using published<sup>8</sup> atomic energy level data. The polynomial representations given in Table 1 are accurate to 1% between 3,000 and 25,000 K.

We find that it is nearly always impossible to measure accurate equivalent widths of atomic absorption lines directly from IUE spectra due to the difficulty inherent in locating the line-free continuum, combined with the masking of the wings of lines by overlapping blended features. Often it is not possible to see more than the core of a line, and the lack of adequate atomic



**Fig. 1** IUE spectra of three stars at the wavelength of the Bi II resonance transition (vertical dashed line). The nearby antimony line is present in the two Hg–Mn stars (a, b) but absent in the normal star (c). Horizontal ticks indicate zero intensity levels for each spectrum; the line-free continuum is not shown for any of the stars. Bismuth is clearly present in HR7775 but absent in the other two stars. a, HR7775, SWP 6885; b,  $\iota$  CrB, SWP 1334; c,  $\nu$  Cap, SWP 1342.

**Table 1** Polynomial approximations for bismuth partition functions

| Ion    | Polynomial approximation (accuracy 1%)                                  |
|--------|-------------------------------------------------------------------------|
| Bi I   | $3.80711 + 0.019893x + 0.0112005x^2 + 0.00062594x^3 - 0.0000126053x^4$  |
| Bi II  | $1.240245 - 0.178508x + 0.0374349x^2 - 0.00154252x^3 + 0.0000250844x^4$ |
| Bi III | $1.955347 - 0.004467x + 0.00338417x^2 - 0.000034814x^3$                 |
| Bi IV  | $1.0005767 + 0.00142463x - 0.00031568x^2 + 0.000015443x^3$              |

$$x = (T, \text{K}/1,000)$$



line lists in the UV means that many blending features are not clearly identifiable. On the other hand, for very strong saturated lines, any blends in the saturated central portion do not affect the calculations as one is observing radiation coming from the boundary layer of the star. In general we feel that abundance estimates based on strong ( $W_\lambda > 100 \text{ m}\text{\AA}$ ) UV lines are more reliable than those based on weak features. We also note that these strong lines lie high on the damping portion of their respective curves of growth, so that knowledge of the microturbulent velocity parameter  $\xi_T$  becomes irrelevant to the abundance problem. Conversely, it is difficult to deduce a meaningful value of  $\xi_T$  from IUE spectra unless it happens to be very large.

Oscillator strengths for Bi II lines have been calculated by Gruzdev<sup>9</sup>; there are no published experimental values. Table 2 lists wavelength,  $\log gf$ , equivalent width and deduced abundance on a logarithmic scale for seven reasonably unblended Bi II lines which we feel can be analysed profitably. The abundances, on the scale  $\log A(\text{H}) = 12.00$ , are given for  $\xi_T = 0$  and  $2 \text{ km s}^{-1}$ ; the results for the five strongest lines are insensitive

**Table 2** Bi II Lines in HR7775

| $\lambda (\text{\AA})$ | $\chi_{\text{lower}}(\text{eV})$ | $\log gf$ | $W_\lambda(\text{m}\text{\AA})$ | $\log A$      |               |
|------------------------|----------------------------------|-----------|---------------------------------|---------------|---------------|
|                        |                                  |           |                                 | $(\xi_T = 0)$ | $(\xi_T = 2)$ |
| 1,436.83               | 0.00                             | -0.60     | 278                             | 6.75          | 6.74          |
| 1,325.46               | 1.64                             | -0.44     | 134                             | 6.59          | 6.56          |
| 1,791.93               | 1.64                             | -0.68     | 86                              | 6.30          | 6.01          |
| 1,902.41               | 2.10                             | -0.35     | 178                             | 6.85          | 6.81          |
| 1,393.92               | 2.10                             | -0.26     | 204                             | 6.93          | 6.91          |
| 1,372.61               | 2.10                             | -0.73     | 157                             | 7.36          | 7.34          |
| 1,787.47               | 4.19                             | -0.19     | 72                              | 6.83          | 6.37          |

to this parameter. The equivalent widths listed are the strengths the lines would be expected to have in the absence of blending features in the spectrum (see Fig. 2a).

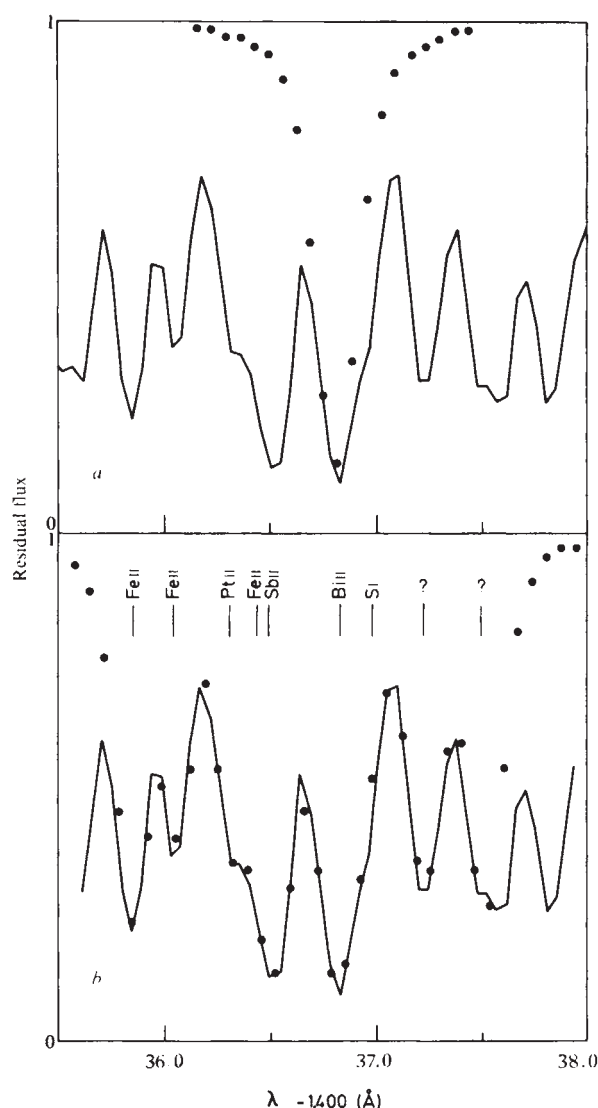
The average abundance for Bi is  $\log A = 6.8 \pm 0.1$  for  $\xi_T = 0$  or  $6.7 \pm 0.2$  for  $\xi_T = 2 \text{ km s}^{-1}$ . This result may be compared with the Solar System abundance<sup>10</sup>  $\log A = 0.8$ ; bismuth is overabundant by a factor of  $\sim 10^6$  in the atmosphere of HR7775. It may be of passing interest that bismuth has only one stable isotope ( $^{209}\text{Bi}$ ) and is the heaviest naturally-occurring stable element in the Periodic Table.

The 12 Hg-Mn stars with which we have compared HR7775 are those listed in Table 1 of ref. 2, plus HR4072 and  $\chi$  Lup. The latter two stars and  $\iota$  CrB are similar to HR7775 in  $T_{\text{eff}}$ ,  $\log g$ ,  $v \sin i$  ( $\leq 2 \text{ km s}^{-1}$ ), and overall composition anomalies<sup>11</sup>, except that they do not have the extreme Ga abundance anomaly present in HR7775, nor do they have the bismuth anomaly. In this sense HR7775 may be said to be a very 'extreme' Hg-Mn star.

Michaud<sup>12</sup> has recently summarized theoretical progress in applying the diffusion hypothesis to the problems posed by the existence of Hg-Mn stars. The theory appears to explain many of the observed anomalies, and their presence only in stars with rotational velocities  $< 90 \text{ km s}^{-1}$ . Because bismuth anomalies have not been previously reported no theoretical calculations for this element have yet been made. However, the fact that  $\iota$  CrB,  $\chi$  Lup and HR4072 are all double-lined spectroscopic binaries<sup>13-15</sup> while HR7775 seems to be a single star may be relevant. Guthrie and Napier<sup>16</sup> have proposed that the presence of a binary companion's illumination can produce atmospheric currents which inhibit diffusion. Michaud quotes unpublished results which suggest that viscosity may reduce this effect considerably but does not eliminate it entirely. Although the true situation remains obscure, these results imply that single stars may have a greater chance of becoming very extreme Hg-Mn objects than binary stars. The strong and so far unique bismuth anomaly found in HR7775 could perhaps support this idea.

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**Fig. 2** *a*, The profile of the Bi II line is shown superimposed on the spectrum of HR7775. The presence of other absorption lines means that the complete profile cannot be observed. *b*, Synthesized spectrum of HR7775 including other lines. The solid line is the observed spectrum, the dots are the synthesized spectrum. The red side of the Bi II line is distorted by a Si I feature. Several unidentified lines are present. In the synthesis calculation these are arbitrarily assumed to be iron lines. All features have been adjusted in strength to fit the observed spectrum, but the primary purpose of this is to simulate their effect on the Bi II line.

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# Disappearing solar filaments and geomagnetic activity

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Early attempts to link solar filaments and their disappearances with geomagnetic activity<sup>1-7</sup> were inconclusive. Joselyn and McIntosh<sup>8</sup> have suggested that some magnetic storms are produced by disappearing filaments. However, we feel that their study included too few cases to establish this link conclusively because, of the 12 possible filament-storm associations shown in their Table 2, they acknowledge that seven are subject to doubt. Therefore we have investigated this problem using a large data sample obtained between 1974 and 1980 inclusive. We found that the average level of geomagnetic activity increases 3-6 days after the disappearance of large solar filaments. We report here that both the magnitude of the disturbance and the delay in its occurrence seem to depend on the filament size. These results support the inclusion of disappearing filaments within techniques that are used for predicting geomagnetic activity.

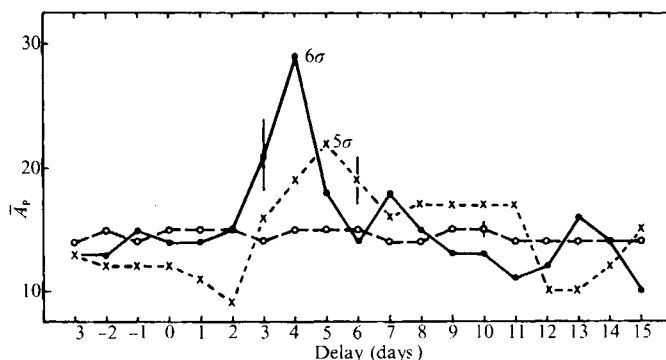
The positions, sizes and times of disappearance of 766 filaments were compiled from daily H $\alpha$  filtergrams produced by the Solar Observatory, Culgoora, New South Wales. Filament disappearances were detected by comparing consecutive daily filtergrams. Therefore the uncertainty in the time of disappearance of each filament was about 1 day. H $\alpha$  filtergrams<sup>9</sup> from Boulder, Colorado, USA, were used to supplement the data where short breaks (~2-3 days) occurred in the Culgoora daily record. Because filaments are structures that are often of great vertical, as well as horizontal extent, their true positions and sizes cannot accurately be determined from their disk projections seen on H $\alpha$  filtergrams. To represent the positions we used the heliographic coordinates of the midpoints of the filaments as seen on the filtergrams. To represent the size we at first (following Joselyn and McIntosh<sup>8</sup>) used the length of the filaments in heliographic degrees. However, we noticed that many filaments of similar length were vastly different in overall area. Furthermore we found, during subsequent analysis, that geomagnetic activity was more strongly correlated with the area of the filament than with its length. Hence, to represent the size we used the corrected area which was defined as follows. The approximate lengths in heliographic degrees of the filaments were determined from the coordinates of their end points. These were multiplied by the average widths which were assumed to be aspect insensitive. The resulting areas were expressed in heliographic square degrees. Filaments at all positions on the disk and with corrected areas greater than 3 square degrees were considered.

To assess the effects of the disappearing filaments on the geomagnetic field the method of superposed epochs was used to determine the average values of the  $A_p$  indices preceding and following times of filament disappearance. The results for filaments in three area ( $A$ ) classes are shown in Fig. 1.

**Table 1** Frequency of occurrence of disturbed geomagnetic conditions following disappearing filaments

| Sample    | No of cases for which $A_p$ exceeded: |      |      |      |
|-----------|---------------------------------------|------|------|------|
|           | 12.6                                  | 25.2 | 37.8 | 50.4 |
| Filaments | 20                                    | 13   | 8    | 8    |
| Control   | 18                                    | 7    | 4    | 2    |

Mean background level of  $A_p$  = 12.6.



**Fig. 1** The results of a superposed epoch analysis showing the average  $A_p$  before and after the disappearance (on day zero) of filaments in three area classes. ●,  $A > 70$  square degrees,  $N = 22$ ; ×,  $40 < A \leq 70$  square degrees,  $N = 35$ ; ○,  $0 < A \leq 40$  square degrees,  $N = 614$ ; where  $N$  is the sample size. Only filaments within the longitude range E65-W65 and the latitude range S65-N65 have been included. The error bars represent twice the r.m.s. fluctuations about the mean background  $A_p$ . The significance of each peak in terms of r.m.s. deviations ( $\sigma$ ) above the mean background level is indicated.

Filaments in the area class  $A > 70$  square degrees were accompanied by a marked increase in the average  $A_p$  between about 3 days and 6 days after their disappearance. The peak value of 29 on the fourth day is approximately 6 r.m.s. deviations above the mean background  $A_p$ . Filaments in the range  $40 < A \leq 70$  square degrees also show a clear correlation with  $A_p$ , with a longer delay. Small filaments for which  $0 < A \leq 40$  square degrees produce no detectable effect on  $A_p$ . It could be argued that the effects shown in Fig. 1 arise from the fortuitous occurrence of one or two large geomagnetic storms after the disappearance of one or two filaments. To show that this is not the case we have determined the values of the  $A_p$  index during the period 3-6 days (inclusive) after the times of disappearance of each of the 22 large filaments ( $A > 70$  square degrees) in our sample. These were compared with the values of  $A_p$  in a control sample consisting of 22 four-day intervals chosen randomly from within the period spanned by the filament observations. The results are shown in Table 1. The first row shows the numbers of filaments that were followed by values of  $A_p$  greater than given thresholds. The second row shows the number of randomly chosen four-day intervals that contained values of  $A_p$  greater than these thresholds. Thirteen filaments were followed by values of  $A_p$  that were greater than twice the mean background level whereas only 7 of the 22 randomly chosen intervals contained values of  $A_p$  greater than this threshold. Further doubling of the threshold increased the disparity between the results for the filament sample and the control sample. Clearly a much higher proportion of filaments is followed by geomagnetic disturbances than would be expected on the basis of chance. Thus we consider the correlation of geomagnetic disturbances with disappearing filaments to be well established.

Most early studies<sup>1-7</sup> were directed towards the identification of filaments (or prominences) with M-regions, the postulated solar sources of recurrent magnetic storms. For this purpose geomagnetic activity was correlated with the times of central meridian passage of the filaments (or the sites of their disappearance) rather than the times of disappearance. While this approach was justified in the search for M-regions it shrouded any effect on the geomagnetic field that was correlated with the times of individual filament eruptions. Hence although a superposed epoch analysis attempting to relate geomagnetic activity with disappearing filaments was performed with a large data sample<sup>7</sup> no significant relationship was detected. Also, little attention was paid to the sizes of the filaments used in the studies. Our results show that only the disappearances of large filaments are followed by clear effects on  $A_p$ . When filaments of all sizes are lumped together the average effect per filament on  $A_p$  is not detectable.

The positive correlation between the average geomagnetic activity and the area of the filaments will be discussed elsewhere<sup>10</sup> where we suggest that it arises for two reasons. First, large filaments may produce more extensive disturbances in the solar wind which have a higher probability of intercepting the Earth. Second, the particle and magnetic flux densities within these disturbances may be greater. Whatever the details of the correlation, it is clear that some disappearing filaments signal the occurrence of geomagnetic disturbances. Therefore, as suggested by Joselyn and McIntosh<sup>8</sup>, they should be incorporated into techniques that are used for the prediction of geomagnetic storms.

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## Apparent correlation of palaeomagnetic intensity and climatic records in deep-sea sediments

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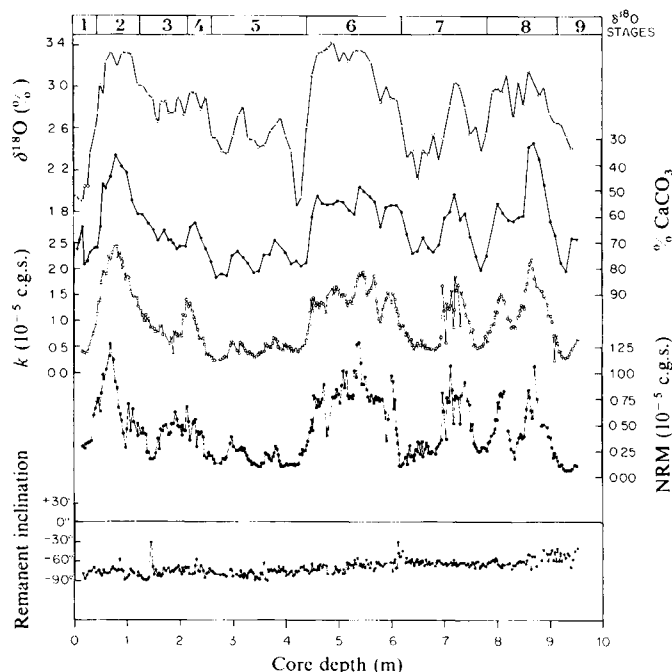
Most reports of a correlation between Pleistocene climate and geomagnetic field intensity rely strongly on the assumption that sediment natural remanent magnetic (NRM) intensity provides a record of geomagnetic field strength and is not sensitive to local changes in properties of the sediment. Critical assessment of relevant data presented here and elsewhere<sup>1,2</sup> from deep-sea sediment cores shows that a pronounced dependence of NRM intensity on sediment composition can occur which implies that this assumption is unlikely to be generally valid. As sediment composition often reflects varying depositional conditions induced by climatic change, the significance of correlations proposed between Pleistocene palaeomagnetism and climatic indicators in deep-sea sediments may be less dramatic than sometimes supposed.

A fundamental relationship between geomagnetic field behaviour and climate change has been suggested, largely on the basis of an apparent correlation in geomagnetic and climatic indices over time scales ranging from historical observatory records of geomagnetic intensity and air temperature<sup>3</sup> to the Pleistocene record of palaeomagnetic and palaeoclimatic data in deep-sea sediments<sup>4,5</sup>. Periods of low (high) geomagnetic intensity have been proposed to correspond to high (low) air temperature or warm (cold) climate. Suggestions for the underlying mechanism have included magnetic field interaction with the atmosphere thereby influencing climate<sup>6</sup>, the distribution of ice affecting core motions and the geomagnetic field<sup>7</sup>, or that there is some common causative agent, for example, variation in the eccentricity of Earth's orbit modulating both climate and the geomagnetic field<sup>8–10</sup>. A hypothetical magnetic trigger has even been entertained to explain climate change on Mars<sup>11</sup>. In view of the lack of consensus on a viable causal mechanism and the complexity of the two systems involved, it seems worthwhile to examine the nature of the reported empirical correlations to ascertain which lines of inquiry are likely to be productive or if they have so little observational basis that they will probably result in unproductive speculation. In this regard, Sternberg and Damon<sup>12</sup> found no statistically significant global

relationship between geomagnetic intensity and climate over the period covered by direct observatory records.

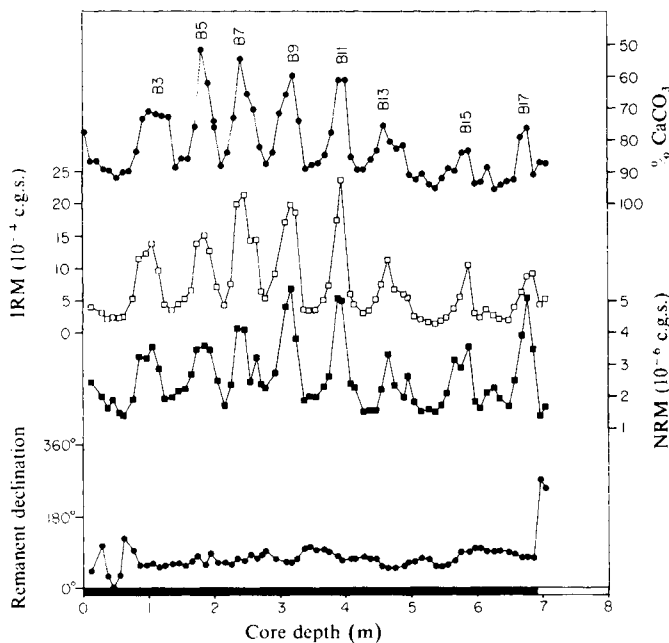
Correlations between geomagnetism and climate over geological timescales are by necessity based on indirect records such as those obtained in continuously deposited deep-sea sediments. Deep-sea core RC11-120 from the southern Indian Ocean (lat. 48°31'S, long. 79°52'E) has one of the most complete and detailed records of climate change available for the past 250,000 yr<sup>13</sup> and proved suitable for magnetic study. The down-core variation of oxygen isotope composition (<sup>18</sup>O) of planktonic foraminifera, carbonate content (CaCO<sub>3</sub>), magnetic susceptibility (*k*) and the intensity of stable NRM and its inclination direction are compared in Fig. 1. The <sup>18</sup>O variation is interpreted to reflect changes primarily in global ice volume and provides a climatic record which can be correlated worldwide<sup>13,14</sup>. The remanent inclinations are generally consistent with expected values for a normal polarity dipole field and show little indication of anomalous field behaviour except possibly for a long-term trend which is not considered here. The striking feature in all these data, however, is the close visual correlation of the <sup>18</sup>O, CaCO<sub>3</sub>, *k* and NRM curves. The significance of these correlations bears directly on the question of geomagnetic-climatic relationships.

Although carbonate deposition is a complicated process with many controlling factors, a close association between carbonate content and climate has been recognized in many Pleistocene deep-sea pelagic sediments from early stages of research<sup>15,16</sup>. In core RC11-120, the high inverse correlation between <sup>18</sup>O and CaCO<sub>3</sub> (*r* = −0.79) indicates that high carbonate contents closely correspond to intervals of low global ice volumes. The same climatic interpretation of carbonate contents can be made



**Fig. 1** Palaeoclimatic, lithological and palaeomagnetic record in deep-sea sediment core RC11-120. Shown from top to bottom are down-core logs of: *a*, oxygen isotope composition of planktonic foraminifera ( $\delta^{18}\text{O}$ )<sup>13</sup>; *b*, calcium carbonate content (% of CaCO<sub>3</sub>, with scale inverted); *c*, magnetic susceptibility (*k*); *d*, intensity of NRM; *e*, the inclination direction of NRM. The intensity and direction of NRM are after alternating field demagnetization at 100 Oe to remove unstable magnetization component. The  $\delta^{18}\text{O}$  values are interpreted to reflect primarily global ice volume and the record is subdivided into the stages shown at the top, odd (even) numbered corresponding to interglacial (glacial) intervals; the age of the stage 1/2 boundary is approximately at 9.4 kyr, stage 5/6 boundary at 127 kyr and the stage 7/8 boundary at 251 kyr<sup>13</sup>.





**Fig. 2** Lithological and palaeomagnetic record in deep-sea sediment core RC11-209. Shown from the top are down-core logs of: a, calcium carbonate content (%  $\text{CaCO}_3$  with scale inverted)<sup>22</sup>; b, IRM; c, intensity of NRM; d, the declination direction of NRM. The NRM and IRM parameters are after alternating field demagnetization at 100 Oe. The 180° shift in declination at 690 cm is interpreted as the Brunhes/Matuyama boundary, age 730 kyr. In this area of the world ocean, high (low) carbonate contents correspond to glacial (interglacial) intervals, for example, the carbonate low labelled B3 correlates to oxygen isotope stage 5 in Fig. 1.

for late Pleistocene sediments from the Atlantic, Caribbean, North Pacific as well as other areas of the Southern Ocean<sup>17,18</sup>.

Factors likely to influence the intensity of NRM are the quantity of magnetic material, typically magnetite, in the sediment and the strength of the geomagnetic field at the time the remanence was acquired<sup>19-21</sup>. The good correlation between NRM intensity and the  $^{18}\text{O}$  curve observed here ( $r = 0.74$ ) may therefore signify a close relationship between geomagnetic field and climate, as has been suggested on the basis of similar comparison, assuming that variations in magnetic mineralogy can be discounted as an important contributing factor. The magnetic susceptibility provides a measure of the concentration of magnetic material and in this core is found to be highly correlated to NRM intensity ( $r = 0.85$ ). The assumption that NRM intensity is simply related to geomagnetic field strength is therefore not supported. Moreover, although other non-calcareous but non-magnetic components are likely to contribute to the total sediment composition, for example biogenic silica and clay minerals, the high inverse correlation between  $k$  and  $\text{CaCO}_3$  ( $r = -0.93$ ) shows that to a good approximation the magnetic material occurs in fixed proportion to the non-carbonate fraction which in turn varies as the complement to the measured carbonate content in these sediments. In such circumstances, variations in magnetic susceptibility can be interpreted to have climatological significance, in an analogous manner to that ascribed to carbonate variations.

On the basis of these relationships, the observed correlation between NRM intensity and climate (for example, the  $^{18}\text{O}$  record) can be largely accounted for as due to an intermediary lithological effect, that is, decreased carbonate contents during glacials result in increased concentrations of magnetic material which in turn contribute to higher NRM intensities. Absence of positive evidence for a correlation of geomagnetic field and climate change is apparent even using a more representative index of relative geomagnetic field strength, obtained by normalizing the NRM intensity of each sample by its magnetic susceptibility. The correlation between this palaeointensity

index (NRM/ $k$ ) and the  $^{18}\text{O}$  climatic curve is negligible ( $r = 0.04$ ).

Another example of how NRM intensity can be more dependent on lithology than geomagnetic field is seen in deep-sea core RC11-209 (lat. 3°39' N, long. 104°4' W). Previous litho-, bio- and magnetostratigraphical studies indicate that this core is representative of the Pleistocene sedimentary history of the equatorial Pacific<sup>22</sup>. Figure 2 shows a comparison of carbonate content, isothermal remanence (IRM, a parameter like magnetic susceptibility which is proportional to content of magnetic material) and NRM intensity values for the upper part of the core, down to the level marked by a 180° shift in remanent declination which is correlated to the Brunhes-Matuyama geomagnetic reversal<sup>22</sup>, now dated at 730,000 yr BP (ref. 23). There is less detail than in core RC11-120 because of a lower deposition rate but the same kind of correlations can be seen over a longer time interval. High carbonate values in RC11-209 again generally correspond to reduced magnetic mineral contents ( $r = -0.93$  for  $\text{CaCO}_3$  versus IRM) which seem to account for reduced sediment magnetizations ( $r = 0.86$  for IRM versus NRM). Even though an oxygen isotope climatic record is not available for core RC11-209, it is well known that high carbonate contents correspond to high global ice volumes or glacial intervals in Pleistocene pelagic sediments from the equatorial Pacific<sup>16,22,24</sup>, exactly opposite to the phase relationship interpreted for carbonate variations and climate in southern Indian Ocean core RC11-120. Therefore, whereas high NRM intensities characterize sediments deposited during glacial intervals in one region (for example, RC11-120), high NRM intensities are associated with sediments deposited during interglacials in another (for example, RC11-209). This is a further indication that the assumption that NRM intensity provides a reliable record of geomagnetic field intensity change is suspect, since geomagnetic intensity and climatic variations are expected to have a similar phase relationship globally. Interestingly, a weak positive correlation ( $r = 0.52$ ) between NRM/IRM and  $\text{CaCO}_3$  is observed in RC11-209 which can optimistically be related to some geomagnetic-climatic dependency or more likely results from an inadequacy in the normalization by IRM to take into account fully the strong lithological effect on the NRM.

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## Intraplate lithosphere strength and heat flow

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The magnitude levels of intraplate stresses and the strength of intraplate lithosphere are critical for understanding the mechanisms of intraplate deformation. The decay of stress in the ductile lower lithosphere and its resulting amplification in the brittle upper lithosphere constitute an important mechanism for providing stress levels in the upper brittle lithosphere sufficient for fracture. Such stress transfer and the subsequent lithosphere deformation are controlled by lithosphere rheology. A model is examined here for the deformation of viscoelastic lithosphere, with temperature- and stress-dependent rheology, subjected to laterally applied stress for different lithosphere thermal structures. The geotherm is found to be of critical importance in determining the strength of the lithosphere and the extent of the resulting lithosphere deformation. For lithosphere with heat flow  $>75 \text{ mW m}^{-2}$ , 0.25 kbar tensile stress is sufficient to cause geologically significant deformation, while for compressional deformation, either greater stress levels or a hotter lithosphere are necessary.

A fundamental property of normal intraplate lithosphere is that it exhibits no significant internal lateral distortion over time periods of the order of tens or hundreds of Myr as testified, for example, by the exactness of fit of the passive continental margins across the Atlantic<sup>1</sup>. This lack of distortion of normal intraplate lithosphere implies that the magnitude of the stresses within continental lithosphere is too low, compared with lithospheric strength, to produce geologically significant lithosphere strains. Conversely, there are certain local tectonic situations within plates where large lateral strains do occur as a result either of larger stress levels or weaker lithosphere. Such intraplate lithosphere deformation includes both extensional fault-bounded basins and rifts and compressional features such as intraplate fold and thrust belts situated at some distance from plate boundaries.

Turcotte and Oxburgh<sup>2</sup> have summarized the mechanisms for generating lithosphere stress. The highest stress available in intraplate regions is probably of the order of 1 kbar or so. Although stresses of this magnitude could fracture the upper part of an elastic lithosphere, they could not fracture it throughout. The ductile nature of the middle and lower lithosphere has been well established from lithosphere flexure studies<sup>3,4</sup> and can also be deduced from experimental work on creep deformation<sup>5-7</sup> and estimates of the geotherm<sup>8,9</sup>. This non-elastic lithosphere can be modelled with either a plastic or viscoelastic constitution. One of the fundamental properties of the lithosphere is that it acts as a stress guide, and it follows that for a plastic or viscoelastic lower lithosphere, stress will decay in the lower lithosphere and be transferred to the purely elastic upper lithosphere, resulting in stress amplification in the upper lithosphere. The process of stress amplification has been investigated for various types of lithosphere and is described elsewhere<sup>10,11</sup>.

The deformation of the lithosphere in response to applied external stress will instantaneously take the form of elastic deformation with strains of the order of 0.01% (Fig. 1a). Creep in the lower ductile lithosphere causes stress decay and associated stress amplification in the upper lithosphere, leading to an increase in the elastic strains there (Fig. 1b). If the stress amplification in the upper lithosphere is sufficiently large, fracture may occur in the topmost brittle lithosphere. Associated stress release further increases the stress levels in the remaining

competent brittle lithosphere. For sufficiently high levels of stress, the whole of the brittle lithosphere may fracture, leading to stress release and transfer to the lower ductile lithosphere. Creep again transfers the stress back to the upper lithosphere where further fracture can occur. A cyclic process of upper lithosphere fracture and lower lithosphere creep then occurs. This process ultimately results in complete failure throughout the competent brittle lithosphere when the stress levels everywhere exceed the failure strength (Fig. 1c). This development is defined as whole lithosphere failure (WLF), after which extensive lithosphere deformation with large horizontal strains take place.

This process has been explored quantitatively using a mathematical model of viscoelastic lithosphere subjected to an applied lateral stress. Power law, temperature-dependent creep corresponding to that of dislocation creep in olivine and granodiorite has been used<sup>6,12-14</sup>. The rheology of the lithosphere is critically important for controlling not only the time taken for brittle failure to occur, but also the rate at which the subsequent deformation takes place.

Lithosphere of initial thickness  $L$  is subjected to an initial applied horizontal stress,  $\sigma_0$ , in the  $x$  direction. Plane strain ( $\epsilon_y = 0$ ) is assumed in the perpendicular horizontal direction and the resulting vertical stress  $\sigma_z$  is assumed to be zero. Conservation of horizontal force and the assumptions that the various layers of the lithosphere are welded to one another provide the additional equations

$$\int_0^L \dot{\sigma}_x dz = 0 \quad \text{and} \quad \frac{d\epsilon_x}{dz} = 0$$

where  $\sigma_x$  is horizontal stress,  $\epsilon_x$  is total horizontal strain and  $z$  is depth.

These equations, together with the constitutive equations for a viscoelastic material, allow the following integral equation to be formulated, giving  $\sigma_x$  as a function of time and depth:

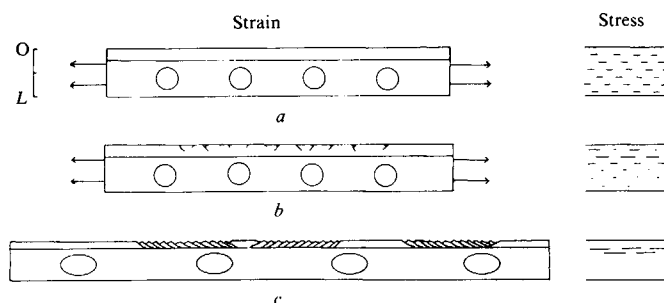
$$\sigma_x = \int_0^t \left( \frac{1}{L} \int_0^L k \dot{\epsilon}_v dz - k \dot{\epsilon}_v \right) dt' - \frac{1}{L} \int_0^L \sigma_x^0 dz + \sigma_x^0$$

where

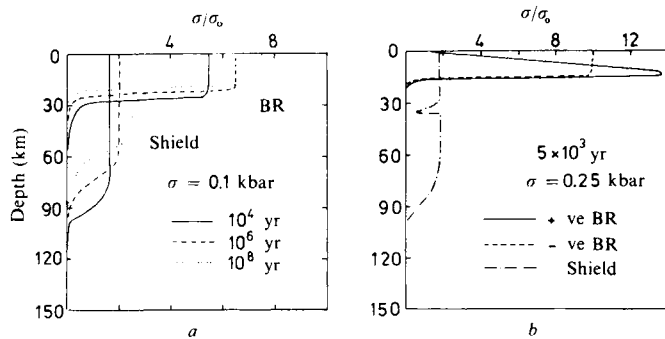
$$k = \frac{E}{1-\nu^2} \quad \text{and} \quad \dot{\epsilon}_v = \frac{\sigma_x(2-\nu) - \sigma_y(1-2\nu)}{6\eta}$$

$E$  is Young's modulus,  $\nu$  Poisson's ratio and  $\eta$  apparent viscosity.

A similar equation exists for  $\sigma_y$ . Fracture has been predicted using Griffiths failure theory<sup>15</sup> and release can be incorporated in the model using the initial stress terms,  $\sigma^0$ . The detailed



**Fig. 1** Lithosphere response to applied horizontal stress. *a*, Elastic response to lithosphere with stresses and strains uniformly distributed with depth through the lithosphere. *b*, Creep in lower lithosphere causes decay of lower lithosphere stresses and associated amplification in upper lithosphere. This may result in fracture of the upper lithosphere. *c*, Extensive stress amplification may lead to complete failure of the brittle upper lithosphere. Whole lithosphere failure then occurs, with large creep strain in the lower lithosphere and cyclic fracturing of the upper lithosphere leading to large strains of the whole lithosphere.



**Fig. 2** Stresses within BR and shield type lithosphere: *a*, with uniform olivine rheology in response to an applied compressive stress of 0.1 kbar at times  $10^4$ ,  $10^6$  and  $10^8$  yr after stress application. *b* With olivine/granodiorite rheology in response to tensile (positive) and compressive (negative) stresses of 0.25 kbar at 5 kyr after application of stress.

formulation of these equations and the stress- and temperature-dependent rheology are described elsewhere<sup>16</sup>. The following parameter values have been used:  $E = 10^5$  MPa;  $\nu = 0.25$ ; Tensile strength = 40 MPa and  $\mu$  (coefficient of friction) = 0.5.

The solutions to these equations are shown in Fig. 2 for continental lithosphere models with both shield and basin and range (BR) type geothermal structure<sup>9</sup>. An initial lithosphere thickness of 150 km is used. Figure 2*a* shows the behaviour of stress for a lithosphere model with uniform olivine rheology subjected to an initial compressive stress of 0.1 kbar (ref. 16). For both shield and BR models stress decays to zero in the lower lithosphere with associated amplification in the upper lithosphere. The stress decay and stress amplification process proceeds more rapidly for the hotter BR lithosphere model; the BR model showing an upper lithosphere amplification of 7 times at  $10^6$  yr against 2 times for the shield model.

Figure 2*b* shows the stress distribution after 5 kyr for an initial applied stress of  $\pm 0.25$  kbar applied to a more realistic lithosphere model with granodiorite rheology above 35 km depth and an olivine rheology below. In the case of the BR lithosphere for tensile applied stress, extensive fracture of the topmost brittle lithosphere, together with the effect of the weaker granodiorite rheology at crustal levels, has resulted in further stress amplification (to 13 times) in the lower brittle lithosphere. The non-fractured part of the lithosphere is extremely thin and WLF is about to occur. However, fracture has not yet occurred for compressive stress. In the case of the shield lithosphere model, no failure has occurred for either tensile or compressive applied stress.

The horizontal lithosphere strain,  $\epsilon_x$ , produced by the composite rheology model is shown in Fig. 3 as a function of time. For 0.5 kbar applied tension, at  $10^6$  yr after the application of stress, large strains of the order of 100% have occurred for the BR model, while those of the corresponding shield model are only of the order of 0.1%. Thus, while the BR type lithosphere undergoes geologically significant deformation, shield type lithosphere does not. Even 1.0 kbar tension does not result in failure for shield. BR lithosphere is considerably stronger in compression than tension; WLF occurring for 0.75 kbar, but not 0.5 kbar. For smaller values of stress, of the order of 0.1 kbar, strains of the order of 0.01% occur which are geologically insignificant. The critical initial stress for extensional deformation of BR type lithosphere is just below 0.25 kbar.

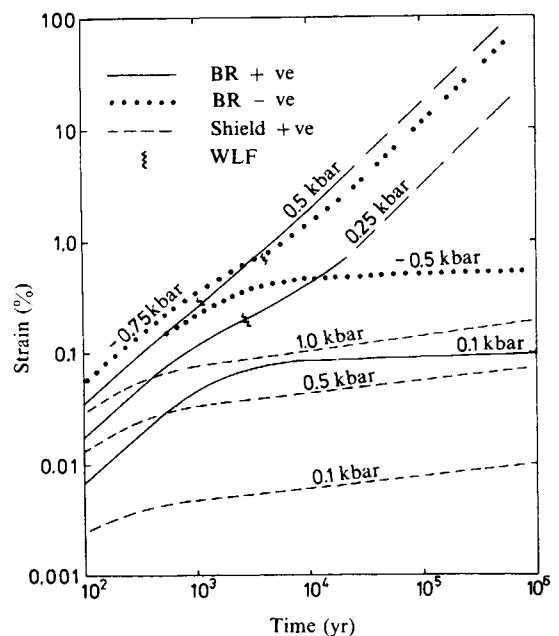
The strain rates indicated in Fig. 3 for those curves where the strain rate has accelerated after WLF are in the range  $10^{-13}$ – $10^{-14}$  s<sup>-1</sup>;  $10^{-14}$  s<sup>-1</sup> is often quoted as a representative geological strain rate<sup>17,18</sup>. An estimate of recent extension in the BR province yields a strain rate of  $10^{-14}$  s<sup>-1</sup> (ref. 19) and for the Kenya rift a rate of  $\sim 10^{-16}$  s<sup>-1</sup> (ref. 20). Figure 3 shows that strain-time curves where WLF has not occurred, and which correspond to average strain rates of the order of  $10^{-17}$  s<sup>-1</sup>, will

not produce significant values of strain even when extrapolated to  $10^8$  yr.

From the relative weakness of BR lithosphere compared with that of shield lithosphere, we may conclude that the most important parameter determining the strength of the lithosphere is its temperature structure. To illustrate this, the lateral deformation of lithosphere having different hypothetical geotherms has been investigated, with results as shown in Fig. 4. The hypothetical geotherm corresponds to that of cooling continental lithosphere and the geotherm is characterized by the resulting surface heat flow. Only lithosphere with heat flow of the order of  $75 \text{ m W m}^{-2}$  or greater will suffer geologically significant lateral deformation when subjected to 0.25 kbar tensile stress. For compressional applied stress, greater stress or higher temperatures are required for significant lithosphere deformation.

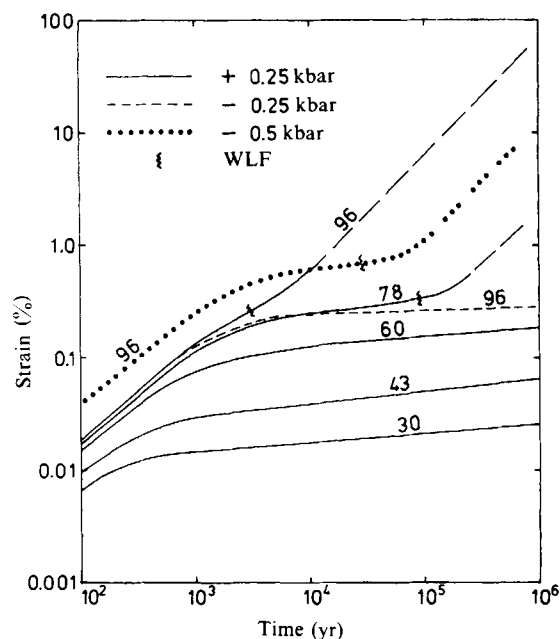
The above models illustrate the importance of thermal structure in determining lithosphere strength. The results of the models might be improved by using a weaker failure law for the upper lithosphere, perhaps incorporating failure along pre-existing zones of weakness. The models also assume that the stress,  $\sigma_0$ , is instantaneously applied to the lithosphere. In reality the stresses would take a finite time, perhaps tens of Myr, to reach a maximum value. To take account of this factor, minor modifications are required of the model which would result in an increase in the time taken for WLF to occur, but would not affect the rate of deformation subsequently. The model will also be affected by alteration of the geotherm due to the effects of extensive horizontal lithosphere strain. This effect would accelerate tensional deformation and retard compressional deformation.

The model may be applied to several different geological situations. Recent tectonic analyses<sup>19</sup> of the BR province suggest that previously unrecognized extensional structures are very significant, and horizontal strains of up to 300% have been suggested. Our model shows that strains of this order could be achieved within a time period of a few Myr, given an initial applied tensile stress of 0.25 kbar. The tensional applied stress would be augmented by the tensile stresses generated by the lateral density contrasts in the mantle and crust associated with the BR plateau uplift<sup>11</sup>. Such stresses also undergo amplification and reach values of around 1 kbar in the upper brittle lithosphere.



**Fig. 3** The variation of horizontal lithosphere strain with time for BR and shield lithosphere in response to different values of tensile (positive) and compressive (negative) initial applied stress.





**Fig. 4** The variation of horizontal lithosphere strain with time, in response to an initial applied stress of  $\pm 0.25$  kbar for hypothetical lithosphere models with different heat flow values (measured in  $\text{mW m}^{-2}$ ). The response to 0.5 kbar compression for  $96 \text{ mW m}^{-2}$  is also shown.

Another application of the model is to the generation of fault-bounded extensional basins like the North Sea Basin. The model proposed by McKenzie<sup>21</sup> for the generation of such extensional basins requires an initial rapid extension causing thinning of the lithosphere, the upwelling of hot asthenosphere and the generation of a thermal anomaly. Our model suggests that rapid extension, as required by the McKenzie basin model, could occur in continental lithosphere with thermal characteristics intermediate between BR and shield. For lithosphere with heat flow of the order of  $80 \text{ mW m}^{-2}$ , an applied tensile stress of 0.25 kbar would be sufficient. Such a heat flow value is today typical of Mesozoic–Upper Palaeozoic post-orogenic areas. According to our model, with an initial tensile stress of 0.25 kbar the lithosphere extension to form the North Sea basin could take place in a few Myr which complies with the requirements of the McKenzie model. Note that the thermal anomaly created by the upwelling of asthenosphere required by the McKenzie model would cause an increase in the rate of stress amplification and would cause the process, once initiated, to accelerate.

The model may also be applied to the recent compressional fold and thrust belts which lie within the Eurasian continental lithosphere north of the Himalayan plate boundary<sup>22,23</sup>. Examples of compressional tectonics many hundreds of kilometres north of the suture can effectively be regarded as intraplate. Assuming any likely thermal structure for this region, intermediate between BR and shield type, it is clear that the model would not produce significant strains under a compressive applied stress of 0.5 kbar. This points either to the existence of much larger stresses in this region, or more likely, to failure zones which are much weaker than those of the model lithosphere.

It is acknowledged that intraplate deformational structures may form for a variety of reasons and that stress levels and temperature structure are not the only factors involved. Localized magmatic processes, for example, will create thermal anomalies which will cause significant local weakening of the lithosphere. Our results suggest that unless unreasonably large stresses are involved, the role of asthenosphere diapirism<sup>24</sup> could be of great importance in initiating failure in all but the hottest type of lithosphere.

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## Cryptocumulate tholeiite as evidence for magma mixing at an intermediate-rate spreading axis

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There is increased interest in possible controls of spreading rate on the geometry and longevity of ocean-ridge magma supply systems<sup>1,2</sup>, and also speculation as to how fractionation processes within these systems may obscure the expression of upper mantle chemistry and isotopic composition in erupted ocean-ridge magmas<sup>3,4</sup>. I report here evidence for reheating and subsequent mixing of plagioclase-cumulitic magma as a result of the influx of primitive picritic magma, at the ('intermediate'-rate) Gulf of California spreading axis, and outline a hypothesis for steady-state fractionation during finite periods of  $\sim 0.35$  Myr. Steady-state systems are expected to become simpler and more long-lived with increasing rates of spreading, thus increasing possibilities for the fractionation of incompatible ('low- $K_D$ ') element ratios.

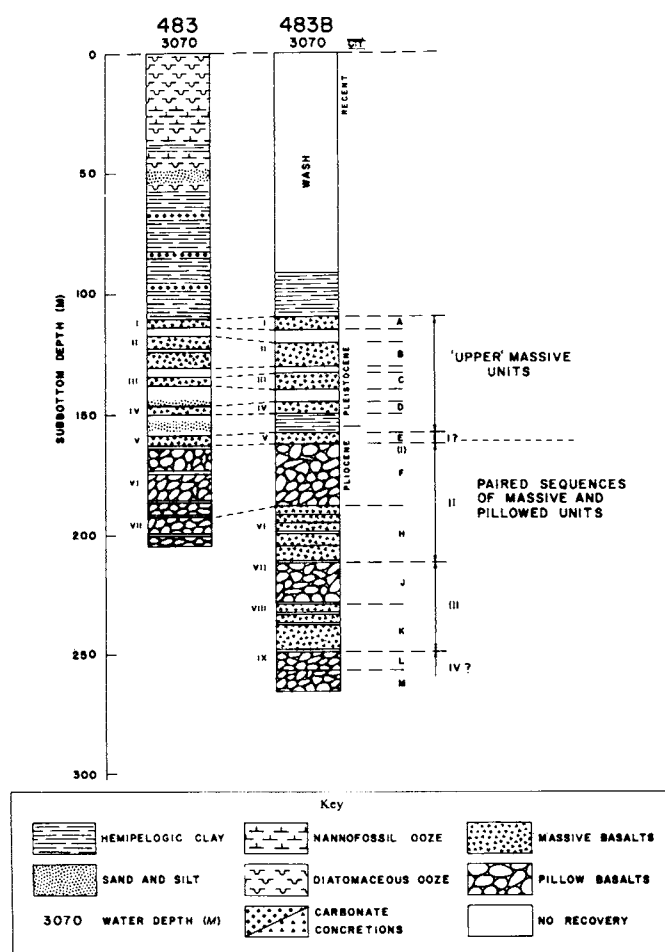
The petrogenetic and chronological relations of ocean basement lava associations clearly have a bearing on the geometric forms of axial magma supply, and the question of whether fractionation occurs in 'closed'- or 'open'-system conditions<sup>3,4</sup>. Kinematic and thermal models predict changes in magma supply as a function of spreading rate, and imply a tendency towards open-system fractionation with increased rates<sup>5,6</sup>. Several

**Table 1** DSDP Site 483: least-squares fractionation solutions for intra-chemical type major element variation (with Zr, Ti and Sr enrichment factors)

| Sample file nos   | Chemical type |        |       | $\Sigma R^2$ | [Zr] <sub>e</sub> | [Ti] <sub>e</sub> | [Sr] <sub>e</sub> |
|-------------------|---------------|--------|-------|--------------|-------------------|-------------------|-------------------|
|                   | % ol          | % plag | % cpx |              |                   |                   |                   |
| a 6200(D) 6190(D) | -1.45         | +5.63  | -0.62 | 0.12         | 0.97              | 0.96              | 1.31              |
| b 5950(C) 6180(C) | -1.36         | +1.63  | -0.41 | 0.29         | 1.03              | 1.03              | 0.99              |
| c 6140(B) 5920(B) | -0.91         | +1.71  | -0.01 | 0.15         | 0.99              | 0.99              | 1.20              |
| d 6090(H) 6100(H) | -0.95         | +0.72  | -0.41 | 0.01         | 1.00              | 1.00              | 1.01              |
| e 6472(H) 6440(H) | -0.51         | +5.55  | -0.20 | 0.24         | 0.96              | 0.95              | 1.02              |
| f 6580(K) 6540(K) | -1.32         | +4.91  | -0.98 | 0.19         | 0.88              | 0.95              | 1.30              |
| g 6660(K) 6710(K) | -0.75         | +5.20  | -0.83 | 0.21         | 0.96              | 0.92              | 1.28              |
| h 6610(K) 6600(K) | -0.99         | +7.11  | -1.35 | 0.16         | 0.90              | 0.85              | 1.37              |

Selected samples are MgO-rich and MgO-poor representatives of the chemical type indicated, or in the case of types H and K, individual massive units comprising these types enrichment factors =  $C_i/C_0$  as defined for Fig. 2. ol, Olivine; plag, plagioclase; cpx, clinopyroxene.

DSDP/IPOD Leg 65 investigated four basement sites (482–485) along a transect of the Gulf of California axis at 23° N (ref. 9), providing detailed information on crust generated at intermediate spreading rates. The gulf is a young rifted basin initiated ~3–4 Myr BP following collision of the Pacific and North American plates. It now forms a northerly extension of the East Pacific Rise (EPR), dilating at a rate of 6.2 cm yr<sup>-1</sup>. Drilled acoustic basement in the segment between the Tamayo and Rivera fracture zones reveals interlayered sequences of massive and pillowed basalts, overlain by thick hemipelagic sediments. The lower horizons of basement sections typically comprise paired sequences of massive and pillowed flows, while upper parts of the sections consist of (somewhat thicker) massive units. The succession is enhanced by high ambient sedimentation rates and seems to reflect sequential eruptive pulses during discrete episodes of crustal accretion<sup>10</sup>. Submersible studies<sup>11,12</sup> of the axis at 21° N indicate an *en echelon* eruptive fissure pattern within the axial rift valley. Sheet flows pond against the rift valley walls up to 1,000 m from their source fissures, while pillowed flows form stubby volcanic ridges along the active zones. Hydrothermal vents are aligned with these ridges. The



**Fig. 1** Basement sections at Site 483, Gulf of California, 23° N, showing lithological units and chemical types (CT) (A–M) from refs 9 and 13. (Chemical types represent compositional/stratigraphic groupings for which complete cross-hole correlation is established.)

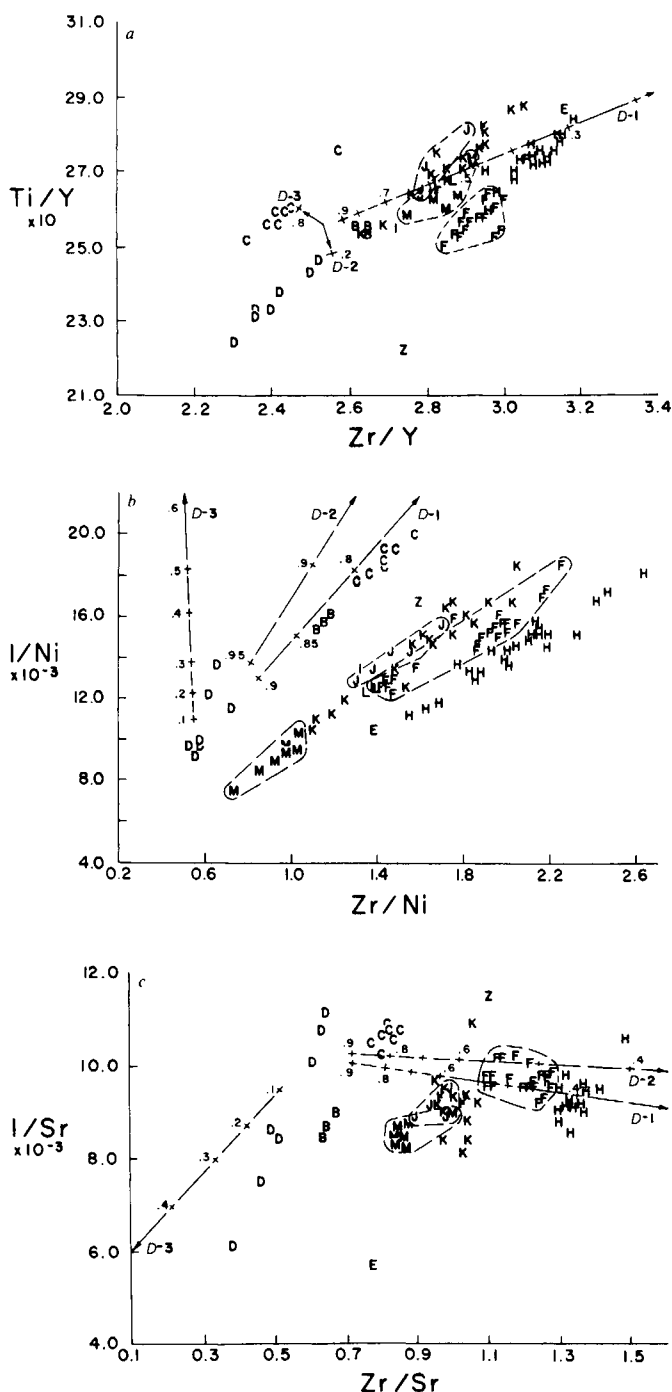
| (ref.)                         | 1<br>(13)         | 2<br>(13) | 3<br>(13) | 4<br>(21) | 5<br>(22) | 6<br>(23) |
|--------------------------------|-------------------|-----------|-----------|-----------|-----------|-----------|
|                                | Wt %              |           |           |           |           |           |
| SiO <sub>2</sub>               | 50.26             | 50.08     | 49.56     | 49.69     | 50.67     | 49.62     |
| Al <sub>2</sub> O <sub>3</sub> | 17.42             | 16.51     | 16.86     | 16.88     | 17.36     | 17.16     |
| FeO*                           | 8.00              | 8.26      | 8.25      | 6.62      | 7.94      | 9.18      |
| MgO                            | 7.76              | 8.86      | 9.76      | 10.16     | 7.31      | 7.16      |
| CaO                            | 12.63             | 12.15     | 12.09     | 14.21     | 12.08     | 12.93     |
| Na <sub>2</sub> O              | 2.40              | 2.49      | 2.09      | 1.58      | 2.54      | 2.32      |
| K <sub>2</sub> O               | 0.04              | 0.05      | 0.05      | 0.11      | 0.24      | 0.04      |
| TiO <sub>2</sub>               | 1.01              | 1.10      | 0.90      | 0.56      | 1.19      | 1.26      |
| P <sub>2</sub> O <sub>5</sub>  | 0.07              | 0.08      | 0.06      | 0.07      | 0.15      | 0.13      |
| MnO                            | 0.16              | 0.16      | 0.17      | 0.12      | 0.14      | 0.17      |
| Total                          | 99.75             | 99.74     | 99.79     | 100.0     | 99.62     | 99.97     |
|                                | Parts per million |           |           |           |           |           |
| Zr                             | 60                | 63        | 117       | 42        | 76        | 42        |
| Y                              | 25                | 26        | 37        | 12        | 26        | 21        |
| Sr                             | 118               | 165       | 174       | 124       | 131       | 105       |
| Ni                             | 109               | 87        | 96        | 145       | 137       | 102       |
| Cr                             | 320               | 301       | 285       | 411       | 294       | 182       |

combined results of drilling and submersible studies in the southern Gulf of California indicate that eruptive pulses form distinctive sequences of massive, followed by pillowed, basaltic lava units. The pillows may represent sporadic late-stage eruptions in any given phase.

Chemical studies of the Leg 65 basalts reflect a series of well defined 'chemical types', each corresponding to a compositional grouping of distinct stratigraphic identity<sup>13</sup>. In several cases, chemical types comprise single massive cooling units. Otherwise, they may comprise several massive units or an uninterrupted sequence of pillow lavas. Paired sequences of pillowed and massive units (at lower horizons, for example, of Site 483, see Table 1) and, in some cases, single massive units (at upper horizons) are interpreted to represent discrete magmatic pulses<sup>13</sup> erupted during a comparatively short time interval from the magma reservoir system. These pulses are characterized by coherent internal variation of major and trace elements, and low- $K_D$  element ratios (for example, Ce/Yb, Zr/Y, Ti/Y). In many cases, however, the relatively extensive variation of the latter precludes a genetic association via simple fractional crystallization, even within a single eruptive pulse<sup>13,14</sup>. In general terms, the Leg 65 variation is intermediate in character between that of 'superfast' (12–17 cm yr<sup>-1</sup>) and 'slow' (1–4 cm yr<sup>-1</sup>) spreading axes<sup>2</sup>, as reflected by the chemical indications of incipient to moderate accumulation of plagioclase (compare ref. 13 with 1, 2).

My purpose here is to direct attention to some additional characteristics of Leg 65 basalts, unrecorded in basalts from either slow- or superfast-spreading axes. Several chemical types at Sites 482, 483 and 485 seem to be 'cryptocumulates', that is, reflect the chemical and mass balance characteristics of strongly plagioclase-cumultic magma, in lavas that are poor in or devoid of phenocrysts. Considering, for simplicity, the Site 483 sections (Holes 483 and 483B; Fig. 1), the major element chemical variation reflects a series of discordant (intra-type) trends superimposed on a more generalized (between-type) tholeiitic trend, resembling that of fast-spreading basalts. In a stratigraphic context (Fig. 1) this variation seems to reflect a repeated sequence of fractionation events acting on magmas already exhibiting a distinct range of variation (~10–5 wt % MgO). Least-squares mass balance solutions for several intra-type variation spectra require addition of plagioclase with concomitant removal of olivine and clinopyroxene, thus reinforcing provisional indications of the major element variation for a sequential series of plagioclase-accumulation events. In general, the Leg 65 basalts range from aphyric to moderately phyrlic. Massive units are mostly aphyric (or nearly so), although

pillow lavas may contain significant phenocryst contents. Paradoxically, variation within massive chemical types reflects markedly stronger chemical indications of plagioclase accumulation than for pillows<sup>13,14</sup>.



**Fig. 2** Site 483 variation, according to chemical type, of: a, Ti/Y versus Zr/Y; b, I/Ni versus Zr/Ni; c, I/Sr versus Zr/Sr (pillow lava chemical types outlined). Deflection of variation from straight line trends is attributable, respectively, to: source-related processes (such as variable partial melting, mantle heterogeneity) and/or steady-state 'open-system' fractionation; mafic phase fractionation; and plagioclase fractionation or accumulation. Straight-line variation for all parameters is consistent with binary mixing (ref. 24). Fractionation trends D-1 to D-3 in these diagrams are calculated according to the Rayleigh equation:  $C_L/C_0 = F^{(D-1)}$  where:  $C_L$  is concentration of element in derivative liquid,  $C_0$  is concentration of element in parent liquid,  $F$  is weight fraction of liquid remaining and  $D$  is the bulk distribution coefficient. For the following hypothetical schemes: D-1, pl: cpx: ol (3:3:1); D-2, pl: ol (1:1); D-3, pl accumulation.

Studies of phenocryst chemistry<sup>15,16</sup> suggest four 'phases' of plagioclase crystallization, reflected by respective phenocryst core compositions: (1) An<sub>92-85</sub>, (2) An<sub>85-78</sub>, (3) An<sub>74-65</sub> and (4) An<sub>74-35</sub>. Experimental data<sup>17</sup> indicate phase (1) compositions to be too An-rich to have equilibrated with their host liquid compositions. This is expressed petrographically by embayed morphologies, resorbed cores and multiple sodic overgrowths<sup>15,16</sup>. Phase (2) plagioclase also reflects resorption and overgrowths, while phases (3) and (4) may result from co-tectitic crystallization before and/or during eruption of the magma. Phase (1) phenocrysts are comparatively rare (~1% modal) and occur sporadically in massive units that are otherwise aphyric (for example, chemical types 483-B, -C, -D, -H, -K; Fig. 1). Pillow lava sequences contain plagioclase of phases (2)–(4), usually accompanied by clinopyroxene, (±)olivine. Phenocryst textures in these are often glomerophytic, although rounded clinopyroxene occurs rarely. In the context of chemical variation and mass balance calculations, these observations imply that where An-rich plagioclase crystallized and accumulated it was subsequently largely resorbed. It is suggested that cryptocumulate lavas result from the accumulation of plagioclase, alone, during temporary stagnation of the supply system, followed by renewed injection of primitive magma. This hypothesis is supported by two lines of evidence. First, the least aphyric cryptocumulates are the most MgO-rich (for example, chemical type 483-D), suggesting maximum thermal input. Second, there is a strong indication of mixing (as opposed to fractional crystallization) from the intra-type major and trace element variation (Fig. 2). Detailed analysis of the chemical variation will be presented elsewhere, but it is noted here that variation between compatible elements Cr, Ni and (in some cases) Sr, and incompatible elements Ti, Zr and Y is linear, in contrast to curved variation paths produced by fractional crystallization.

The most convincing evidence for plagioclase accumulation, mixing and reheating occurs in chemical type 483-D, a thick massive unit in the upper part of the Site 483 section. Analyses of cryptocumulates from this unit are compared with coarsely plagioclase-phyric lavas sampled from slow-spreading Atlantic crust (Table 2). Despite their petrographic contrasts these samples reflect remarkably similar chemistries, especially with regard to their CaO Al<sub>2</sub>O<sub>3</sub> and Sr contents. As cryptocumulate magma seems to be absent from fast-spreading axes, and there is clear petrographic expression of accumulated plagioclase in slow-spreading basalts, these liquid compositions and the processes they reflect may be highly characteristic of intermediate-rate spreading axes. They should therefore be carefully distinguished from primary tholeiite melts or derivatives of these resulting from the fractionation of olivine. From a phase equilibrium standpoint, cryptocumulates will be unequivocally characterized by their confinement at low pressures to the plagioclase crystallization field, in contrast to the cotectic (or near cotectic) behaviour generally observed in non-cumulate basalts.

It seems that magma supply maxima are probably time-transgressive along the Gulf of California axis, longitudinal distances between axial highs and lows at any one time being ~90 km (ref. 10). Lewis<sup>10</sup> invoked a propagating magma source as the cause of this variation and calculated a longitudinal eruptive migration rate of ~50 cm yr<sup>-1</sup>, corresponding to an average periodicity of 0.35 Myr. These considerations and the fractionation mechanisms interpreted from Leg 65 sections strongly suggest a variable magma supply rate, both from the upper mantle to the axial reservoir system, and from the latter to the ocean floor. If several magma pulses contribute to single active episodes (that is, correspond to Lewis's estimates for average periodicity) the eruptive history of such an episode may reflect a short-term steady state, if this is not a contradiction in terms. The geochemical effects of such processes are being modelled numerically (see ref. 4), assuming build-up of the Leg 65 sections during a single episode, and given the excellent constraints of eruptive chronology, volume



relations and chemical affinities in the southern Gulf of California<sup>9-16</sup>.

I tentatively conclude that fractionation of magma beneath the Gulf of California spreading axis is neither a continuous steady-state process nor a simple sequence of discrete closed-system events. It probably involves several separate systems, each representing a steady state during its own finite existence. Cryptocumulate magma seems to characterize this regime and can be catalogued with other effects resulting from partial, complete or delayed mixing in the axial supply system<sup>18-20</sup>.

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## Aminostratigraphy and faunal correlations of late Quaternary marine terraces, Pacific Coast, USA

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Recent studies using the extent of racemization of amino acids to date fossil mollusc shells in the Arctic<sup>1</sup>, the British Isles<sup>2</sup> and on the Atlantic<sup>3,4</sup> and Pacific<sup>5-13</sup> coasts of North America have relied mainly on theoretical kinetic models of racemization. Ages generated in this fashion are highly model dependent and require estimates of integrated long-term diagenetic temperatures. We present here an alternative, empirical approach to aminostratigraphy in which we plot amino acid enantiomeric ratios versus latitude (for localities along the Pacific coast of the United States), and generate isochronal correlations by connecting data points of geographically proximal localities that have similar D:L ratios and zoogeographic aspect. Isochrons are calibrated at a few localities by independent radiometric dates. The diagenetic temperature effect on racemization is reflected in the slope of the isochrons, but the need to quantify temperature is eliminated.

Amino acid dating is based on the almost exclusive occurrence in living protein of amino acids in the L-enantiomeric configuration. During diagenesis these amino acids undergo racemization, a spontaneous and reversible chemical reaction in which the L-enantiomers convert to an equilibrium mixture (usually 1:1) of D- and L-enantiomers. The extent of racemization, expressed as a ratio (D:L) of the two enantiomers, is mainly a function of reaction time and of ambient diagenetic temperature. Amino acids in fossil shells exposed to ground temperatures typical of the Pacific coast of the United States reach racemic equilibrium in 1-2 Myr (refs 2, 5, 6). Limited laboratory<sup>14</sup> and geological<sup>5,6,15-18</sup> data demonstrate that racemization (bound + free fractions) in shells follows quasi-exponential kinetics; that is, a rapid initial rate is followed by a significantly slower and decreasing rate<sup>5,6,10,15,16</sup>.

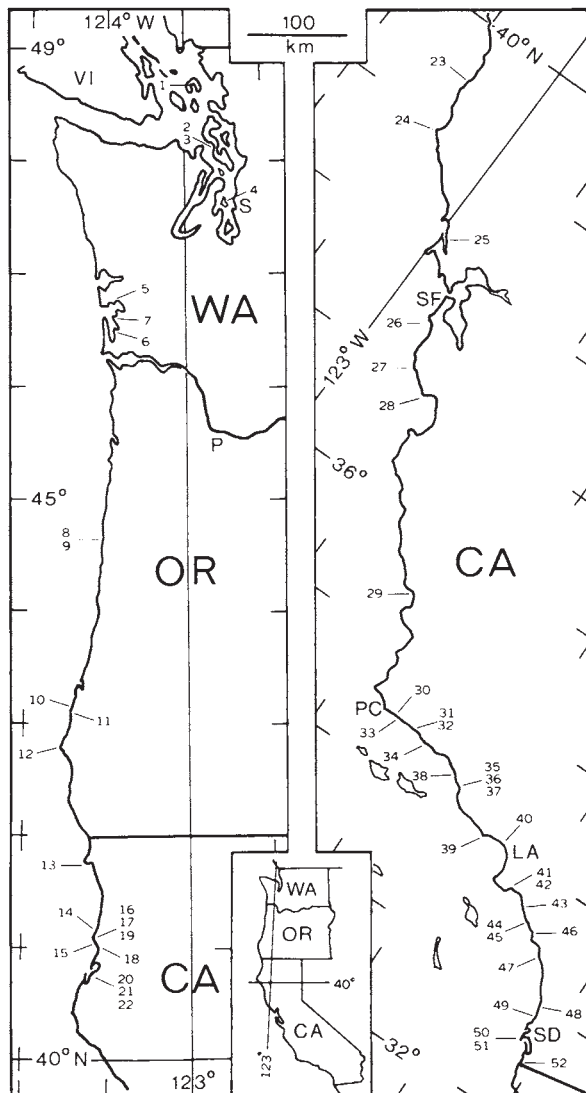
We have determined the enantiomeric ratios of six amino acids (leucine, glutamic acid, proline, phenylalanine, alanine and valine) in more than 500 samples from ~150 Pacific coast localities. However, because different amino acids racemize at different rates and follow different apparent kinetics in different molluscan genera<sup>5,6,16</sup>, all of our data are not directly comparable. For illustrative purposes, we will discuss only leucine data from the genus *Saxidomus*, a thick-shelled bivalve mollusc that is present at about one-third of our localities and which normally yields reproducible data<sup>5,7</sup>. For localities at which *Saxidomus* does not occur, ratios from other bivalve genera (for example, *Protothaca*, *Macoma*) can be converted to equivalent *Saxidomus* values using regression analysis<sup>5,6,16</sup>.

Most of our samples were taken from the hinge or nymph region of the shell and were prepared using techniques summarized in ref. 5. Gas chromatography of the total amino acid residue (free + hydrolysed fractions) was performed using *N*-trifluoroacetic acid-(+)-2-butyl derivatives and stainless steel and glass capillary columns coated with OV225 and Carbowax 20M. Locality data and D:L ratios for most of the localities shown in Fig. 1 are given in refs 5, 7.

Our approach to amino acid geochronology is based on the following assumptions: (1) emergent marine terraces are cut during episodic sea-level high stands<sup>15</sup> whose ages correspond to those of well dated terrace levels on coral-bound tropical islands (for example, Barbados, New Guinea), and with correlative peaks of the marine oxygen isotope record; (2) the zoogeographic aspect of marine invertebrate faunas can be used for correlation purposes; warm-water faunas reflect warmer marine conditions during the high stand of early stage 5 and cool-water faunas reflect the relatively lower high stands of middle-to-late stage 5 and stage 3; (3) radiocarbon ages of Holocene emergent terraces and uranium-series ages of corals from late Pleistocene terraces in southern California are correct; (4) proximally located terrace deposits have experienced similar, but not necessarily identical, long-term average temperatures; (5) the modern latitudinal temperature gradient along the coast is roughly similar to that of the recent geological past.

Two important control localities for calibrating our chronology are Cayucos (Fig. 1, locality 29) and the Nestor Terrace in San Diego (locality 50). These are the only two localities on the mainland coast of California with unambiguous 120,000-130,000-yr BP U-series dates on corals<sup>19</sup>, although similar U-series dates on molluscs exist for other southern California localities<sup>20</sup>. A global sea-level high stand at this time is documented worldwide<sup>21,22</sup>, and is correlative with substage 5e of the marine oxygen isotope record<sup>23,24</sup>. Substage 5e represented a period of climatic amelioration that is documented by poleward latitudinal changes in marine invertebrate distributions in the oceans<sup>25,26</sup>, as well as corresponding floral (pollen) changes on the continents<sup>27,28</sup>. By the onset of substage 5d, global climate had begun to deteriorate rapidly, and sea levels never again reached the substage 5e level. If successively warmer and cooler marine conditions prevailed along the Pacific coast during stage 5, such changes should be identifiable in the fossil record. In fact, the early stage 5 molluscan faunas from Cayucos and the Nestor Terrace both contain extralimital

southern species<sup>29,30</sup>, indicative of warmer coastal marine conditions at that time. Likewise, faunas from late stage 5 terraces, such as the Bird Rock Terrace (below the Nestor Terrace) in San Diego, contain extralimital northern (that is, cool-water) species and, notably, no warm-water southern species<sup>30</sup>.



**Fig. 1** Pacific coast of Washington, Oregon, and California showing fossil localities studied. Locality identification numbers (see below) correspond to numbered data points in Fig. 2. Locality data for most sites are given in refs 5, 7. Localities are: 1, Orcas Island; 2, 3, Whidbey Island (data from refs 35 and 11, respectively); 4, Bainbridge Island; 5, Willapa Bay, north shore; 6, Lynn Point (data from ref. 8); 7, Bay Center; 8, Hinton Point, Yaquina Bay; 9, Newport jetty; 10, Fivemile Point; 11, Coquille Point, Bandon; 12, Cape Blanco; 13, Crescent City (Battery Fm.); 14, Big Lagoon; 15, Moonstone Beach; 16, Luffenholtz Creek; 17, Trinidad Head; 18, Crannell Junction; 19, Elk Head; 20–22, Eureka-Fields Landing area; 23, MacKerricher State Park; 24, Point Arena; 25, Tomales Bay (Millerton Fm.); 26, Half Moon Bay (data from ref. 36); 27, Point Año Nuevo; 28, Santa Cruz; 29, Cayucos; 30, Alegria Canyon; 31, 32, Arroyo Hondo; 33, west of Gaviota; 34, Goleta; 35–37, Ventura oil field; 38, Punta Gorda; 39, Point Dume; 40, Pacific Palisades; 41, 42, San Pedro (San Pedro Fm., 41; Palos Verdes Sand, 42); 43, Huntington Beach; 44, 45, Newport Beach; 46, Laguna Beach; 47, San Onofre; 48, Solana Beach; 49, Torrey Pines State Park; 50, 51, Point Loma (Nestor Terrace, 50; Bird Rock Terrace, 51); 52, international-border locality. Abbreviations for points of reference are: VI, Vancouver Island; S, Seattle; P, Portland; SF, San Francisco; PC, Point Conception; LA, Los Angeles; SD, San Diego. Abbreviations for states are: WA, Washington; OR, Oregon; CA, California.

Terrace pairs with a warm-water fauna on the upper terrace and a cool-water fauna on the lower are known also in southern California (for example localities 31, 32; in preparation), and on the outer coast of central Baja California, Mexico<sup>12</sup>.

Figure 2 shows *Saxidomus* leucine D:L ratios versus latitude for 52 fossil localities from the Pacific coast of California, Oregon and Washington (lat. 32.5–49° N). The isochrons in Fig. 2 are secondarily smoothed curves generated after connecting data points of geographically proximal localities with similar D:L ratios and similar faunal-temperature (zoogeographic) aspect. (Temperature aspect of a faunal assemblage is determined by the area of overlap of the modern geographical ranges of its component species.) Absolute age control for the early stage 5 isochron in Fig. 2 is provided by the U-series dates for the Cayucos and Nestor Terrace localities. Additional control at other localities is provided by various radiometric, zoogeographic, eustatic, tectonic or geomorphic evidence. The D:L ratios for the two U-series-dated localities fall slightly below most of those in southern California that we believe to represent early stage 5 on the basis of their warm-water faunas. Only one *Saxidomus* was available from each of these two localities, and the D:L ratios may not represent the mean values of a larger population. For instance, seven *Protothaca* D:L ratios for the Nestor Terrace in San Diego regress to an equivalent mean *Saxidomus* value of 0.505 (J.F.W., unpublished), compared with the actual single *Saxidomus* ratio of 0.48. The higher ratio would place the Nestor Terrace data point well within the southern California cluster.

It might be argued, however, that the southern California data points above those of Cayucos and the Nestor Terrace may represent an earlier sea-level event than that which occurred ~120,000 BP. There is evidence, for instance from U-series-dated coral reefs in New Guinea, Atauro, Hawaii and other regions<sup>22,31,32</sup>, that emergent terraces identified with oxygen-isotope substage 5e may actually represent two discrete sea-level events, the first culminating about 135,000 BP, followed by a minor regression and subsequent rise 125,000–118,000 BP (ref. 31). Isotopic evidence from New Guinea also suggests that marine temperatures were slightly warmer (1.7–2.5 °C) during the earlier 135,000 BP level than during the subsequent 120,000 BP level<sup>32</sup>. Some of the localities in southern California that plot in the upper part of our early stage 5 cluster (Fig. 2) also have warmer-water molluscan faunas than those found at Cayucos and the Nestor Terrace, which, however, are open-coast localities. Additional data are needed before the precise ages of these localities can be resolved.

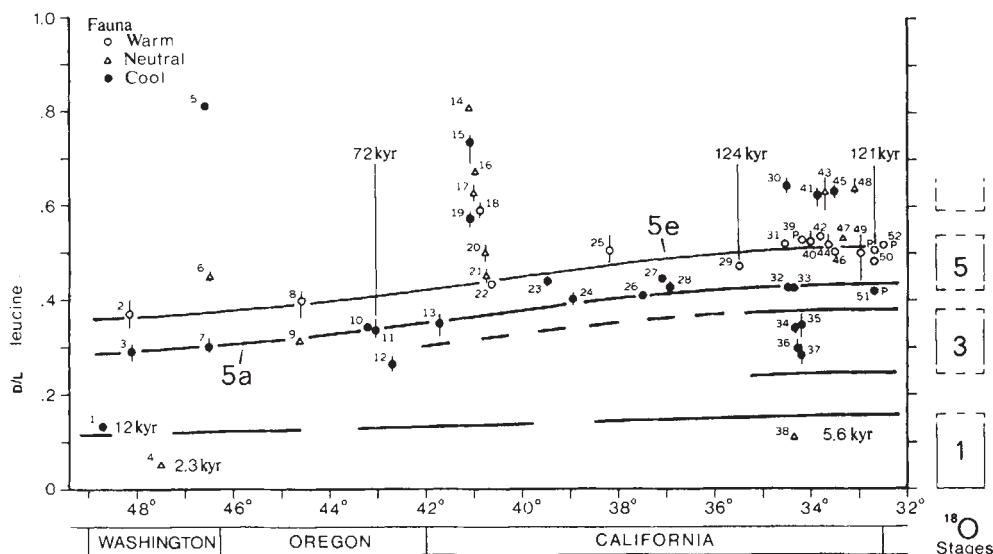
Warm-water fossil faunas from California are rare north of Point Conception. However, the substage 5e isochron can be extrapolated as far north as Puget Sound assuming an effective diagenetic temperature gradient similar to that along the coast today<sup>9</sup>. This extrapolation is supported by both faunal and amino acid data from several localities, including one at Yaquina Bay, Oregon (locality 8), at which 16% of the identifiable molluscs are extralimital southern species<sup>7</sup>.

Absolute age control for the late stage 5 (substage 5a) isochron in Fig. 2 is provided by an apparent <sup>230</sup>Th/<sup>234</sup>U age of 72,200 ± 5,300 yr on solitary corals from the Whiskey Run Terrace in Bandon, Oregon [locality 11; date by T.-L. Ku, unpublished (note however that <sup>231</sup>Pa/<sup>235</sup>U > 1)]. The interpolative isochron generated by connecting data points of proximally located localities with cool-water faunas and similar D:L ratios is roughly parallel to, but lies somewhat below, the early stage 5 curve in Fig. 2. Cool-water, outer-coast faunas that lie along this curve and that are here assigned to late stage 5 differ significantly from early stage 5 faunas, especially north of Point Conception, and are characterized by the presence of 10–20% extralimital northern species<sup>7</sup>. The late stage 5 curve can be extrapolated for localities as far north as Puget Sound without any apparent problems.

Although most of the data points representing cool-water stage 5 faunas lie close to the late stage 5 curve, two do not. The first, that for MacKerricher State Park (locality 23), plots



**Fig. 2** Mean leucine enantiomeric ratios (D:L) versus latitude for localities shown in Fig. 1 (numbered data points correspond to identically numbered localities). Enantiomeric ratios are mostly from refs 5, 7 (except as noted in Fig. 1), and are from the bivalves *Saxidomus giganteus* (Deshayes) and *Saxidomus nuttalli* Conrad, two species known to have similar racemization kinetics<sup>16</sup>. Localities with faunas that indicate oceanic conditions warmer than their modern latitudinal equivalents are shown by open circles, cooler-than-modern conditions by closed circles. Faunas with a modern (neutral) temperature aspect are shown by triangles, as are localities that have low-diversity faunas (containing neither warm- nor cool-water species), or for which we lack faunal data. Probable correlation of amino acid data with oxygen isotope stages is shown in right column. Boundary lines for isotopic stages 1 and 3 are approximations only and may change with additional data. Isochrons for early and late stage 5 localities (labelled 5e and 5a, respectively) are secondarily smoothed curves generated after connecting data points of geographically proximal localities with similar D:L ratios and similar zoo geographic aspect. Substage 5e localities are characterized by the presence of extralimital southern (warm-water) species in their faunas; substage 5a faunas by extralimital northern (cool-water) species. Absolute age control is provided by radiocarbon dates on molluscs (localities 1, 4, 38), and by U-series dates on solitary corals (localities 11, 29, 50). Vertical lines through data points indicate range of data, most of which represent two to four analyses. P indicates a *Protothaca* D:L ratio regressed to an equivalent *Saxidomus* value. Ranges of approximate mean annual temperatures for regions on the Pacific coast are: 8–10 °C for 49–45° N; 10–12 °C for 45–39° N; 12–14 °C for 39–35° N; 14–16 °C for 35–32° N.



close to the warm-water early stage 5 curve, but is assigned a middle-to-late stage 5 age on the basis of: (1) coexisting D:L ratios from the bivalves *Protothaca* and *Macoma*, which indicate that the single *Saxidomus* ratio may be too high<sup>16</sup>, and (2) the cool-water aspect of the fauna, which is zoogeographically equivalent to that of Puget Sound<sup>7</sup>. The second locality, at Cape Blanco, Oregon (locality 12), may represent an early stage 3 terrace, although the cool-water fauna there is not zoogeographically distinguishable from those of late stage 5 localities on the southern Oregon or northern California coasts<sup>7</sup>.

Just as early and late stage 5 faunas can be separated on zoogeographic means, faunas determined to be older or younger than stage 5 on the basis of higher or lower D:L ratios, or by the presence of biostratigraphically important species, can also be distinguished. For instance, younger, stage 3, faunas from the Santa Barbara–Ventura coast in southern California (localities 34–37) have a cooler-water aspect than do nearby late stage 5 faunas (localities 32–33), whereas stage 1 (Holocene) terrace faunas have a modern water-temperature aspect<sup>15</sup>. The geological youth of the post-stage 5 localities is supported by geomorphic and radiometric evidence<sup>6,15,33</sup>. For older, more highly racemized samples, we also assume that differing cool- and warm-water faunas indicate noncontemporaneity, even though the extent of racemization may not appear significantly different, perhaps due to slight variations in their physical or chemical environment over longer periods of time, or to the flattening of the racemization curve at higher D:L ratios. At present, however, too few amino acid or faunal data exist to allow confident long-range correlation of pre-stage 5 terraces along the Pacific coast of North America. Amino acid age estimates for older localities must still be determined using kinetic models of racemization<sup>5,17,34</sup>. Nevertheless, the use of faunal evidence in conjunction with plots of amino acid D:L ratios versus latitude, such as shown in Fig. 2, seems to provide a simple and reliable means of refining aminostratigraphic age estimates and of correlating late Quaternary marine terraces with well dated worldwide climatic and sea-level events.

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## Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall

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Acid rain commonly has high concentrations of dissolved  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Sulphuric and nitric acids are usually considered to be the acidic components, whereas ammonium has a tendency to increase the pH of rainwater<sup>1</sup>. Ammonium can be transformed to nitric acid in soil but this source of acidity is generally less important than wet and dry deposition of free acids<sup>2,3</sup>. Here we describe the occurrence of high concentrations of ammonium in canopy throughfall (rainwater falling through the tree canopy) and stemflow in woodland areas in the Netherlands, resulting in acid inputs to soils two to five times higher than those previously described for acid atmospheric deposition<sup>2-5</sup>. The ammonium is present as ammonium sulphate, which probably forms by interaction of ammonia (volatilized from manure) with sulphur dioxide (from fossil fuels), on the surfaces of vegetation. After leaching by rainwater the ammonium sulphate reaching the soil oxidizes rapidly to nitric and sulphuric acid, producing extremely low pH values (2.8–3.5) and high concentrations of dissolved aluminium in the non-calcareous soils studied. Deposition of ammonium sulphate on the surfaces of vegetation and its environmental consequences are probably most important in areas with intensive animal husbandry.

Two areas were studied: location 1 (Fig. 1) is a 3.2-hectare woodland area with both calcareous and acidic (pH 4) soils, dominated by oak (*Quercus robur*) and birch (*Betula pendula*). The vegetation has remained undisturbed for the past 30 yr. The land use of the region is intensive grass production for silage (75% of the area), and scattered woodlands (25%) similar to the studied area. Location 2 is a woodland area with an acidic sandy soil (inland dunes, pH 4) dominated by Scots Pine (*Pinus sylvestris*). It is part of a 2,000-hectare nature reserve area that mainly consists of heathland. Land use in the surrounding farmland is the same as in location 1. The collecting devices for throughfall and stemflow were sited within 50 m of intensively managed farmland at location 1; there was 100–400 m of woodland between the collecting apparatus and the nearest farmland at location 2.

At location 1, rain (from outside the woodland) and throughfall were collected via 400 cm<sup>2</sup> polythene funnels into dark 5-l polythene bottles. Six throughfall collecting devices were placed at the apices of a randomly located hexagon of side 5 m in each of four 10 × 20 m sites within the woodland, about 150 m from each other. The bottles were sampled fortnightly. Rain samples were collected in duplicate bottles, and throughfall samples from adjacent collecting devices were pooled to give 12 replicates. Samples were analysed immediately for pH, electrical conductivity, organic carbon and all major inorganic ions, including  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  (by ion chromatography),  $\text{HCO}_3^-$  (from pH and total inorganic carbon by IR analysis) and  $\text{NH}_4^+$  (colorimetrically using Nessler's reagent)<sup>6</sup>. At each site, stemflow was intercepted on six randomly chosen trees by silicone-sealed polythene collars and collected in 60-l dark

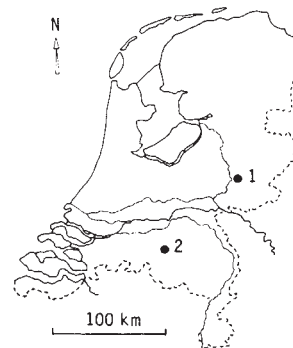


Fig. 1 Map of The Netherlands showing the locations of the areas studied.

polythene bottles. Again, samples from adjacent pairs of bottles were pooled to give 12 replicates, and these were analysed fortnightly.

Preliminary work at location 1 in 1980 showed that the predominant ions in throughfall and stemflow were  $\text{NH}_4^+$  and  $\text{SO}_4^{2-}$  with concentrations far exceeding those in rainwater. Continuous monitoring since January 1981 has shown that the concentrations of  $\text{NH}_4^+$  and  $\frac{1}{2}\text{SO}_4^{2-}$  in individual samples were almost equal and showed a strong correlation with  $r = 0.91$  and  $0.92$  for throughfall and stemflow, respectively: mean values  $\sim 0.6 \text{ mmol l}^{-1}$  (throughfall) and  $2.5 \text{ mmol l}^{-1}$  (stemflow) (Table 1). The volume-weighted mean ammonium concentrations in throughfall and stemflow were respectively 4 and 18 times higher than in rainwater. Although the Nessler assay for ammonium is subject to several sources of interference, such as pollen and formaldehyde<sup>7,8</sup>, the close correspondence of sums of equivalent concentrations of cations and anions, and the similar results at location 2 (where ammonium was assayed by means of the Berthelot reaction), show that these sources of errors were small.

At location 2 a reconnaissance sampling programme was started in 1981. Sampling procedures were similar to those at location 1, but the sampling intensity was reduced: single monthly samples of rainwater and stemflow and duplicate monthly samples of throughfall were obtained. The chemical analyses are described elsewhere<sup>9</sup>. Here also, the concentrations of  $\text{NH}_4^+$  and  $\frac{1}{2}\text{SO}_4^{2-}$  in individual samples were about equal (with  $r = 0.82$  and  $0.98$  for throughfall and stemflow, respectively), and of the same order as those at location 1 (Table 1). The mean concentrations of  $\text{NH}_4^+$  in throughfall and stemflow were, respectively, about 5 and 31 times higher than in incident rainwater.

The  $\text{NH}_4^+$  concentrations in throughfall reported here are 3–20 times higher than those reported for forests in the USA, Sweden, FRG and England<sup>5,10–12</sup>; they are unlikely to have resulted from leaching of leaves and bark because higher  $\text{NH}_4^+$  levels are observed in winter, when the trees are inactive, than in summer. These seasonal trends are consistent with those from all but one (Rotterdam) of the nine monitoring stations for rainfall in The Netherlands<sup>9</sup>. The high ammonium concentrations in throughfall and stemflow are probably due to dry deposition on vegetation surfaces of ammonia volatilized from liquid domestic animal manure which is sprayed on to pastures mainly in winter and early spring. Spreading liquid manure commonly leads to  $\text{NH}_3$  volatilization losses in the order of several hundreds of kg of nitrogen per hectare per application<sup>13,14</sup>, leading to contamination of rain in the vicinity<sup>15</sup>. We estimate nitrogen inputs from throughfall plus stemflow to be, respectively 64 and 63 kg hectare per yr at locations 1 and 2. These values are compatible with an agricultural origin of the  $\text{NH}_4^+$  and are about an order of magnitude greater than those estimated for remote deciduous forests in Massachusetts, USA<sup>16</sup>.

**Table 1** Chemical composition of rain, throughfall and stemflow water and associated fluxes in the studied areas in 1981

|                                                        | Location 1 |               |                 | Location 2 |             |          |
|--------------------------------------------------------|------------|---------------|-----------------|------------|-------------|----------|
|                                                        | Rain       | Throughfall*  | Stemflow*       | Rain       | Throughfall | Stemflow |
| Volume-weighted concentration (mmol l <sup>-1</sup> )† |            |               |                 |            |             |          |
| NH <sub>4</sub> <sup>+</sup>                           | 0.11       | 0.47 ± 0.1    | 1.99 ± 0.51     | 0.09       | 0.45        | 2.79     |
| NO <sub>3</sub> <sup>-</sup>                           | 0.053      | 0.16 ± 0.02   | 0.22 ± 0.09     | 0.05       | 0.14        | 0.45     |
| 1/2 SO <sub>4</sub> <sup>2-</sup>                      | 0.13       | 0.50 ± 0.1    | 2.06 ± 0.55     | 0.12       | 0.49        | 2.92     |
| H <sup>+</sup>                                         | 0.031      | 0.030 ± 0.009 | 0.0035 ± 0.0006 | 0.057      | 0.044       | 0.55     |
| Mean pH                                                | 4.51       | 4.54          | 5.46            | 4.29       | 4.52        | 3.47     |
| Fluxes (kmol km <sup>-2</sup> yr <sup>-1</sup> )       |            |               |                 |            |             |          |
| NH <sub>4</sub> <sup>+</sup>                           | 104        | 303 ± 67      | 44 ± 24         | 77         | 328         | 16       |
| NO <sub>3</sub> <sup>-</sup>                           | 50         | 104 ± 11      | 7.2 ± 6.3       | 43         | 100         | 2.7      |
| 1/2 SO <sub>4</sub> <sup>2-</sup>                      | 123        | 324 ± 69      | 47 ± 23         | 103        | 355         | 17       |
| H <sup>+</sup>                                         | 29         | 19.4 ± 6.0    | 0.09 ± 0.05     | 49         | 32          | 3        |

For location 2, fluxes are order of magnitude estimates.

\* Mean ± s.d. for 12 pairs of collectors.

†  $\sum_{i=1}^N C_i \times F_i / \sum_{i=1}^N F_i$  where  $C_i$  = the concentration of the  $i$ th sample and  $F_i$  = the water flux associated with that sample.

Part of the ammonia may be deposited from the atmosphere on plant surfaces as solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, (NH<sub>4</sub>)<sub>3</sub>H(SO<sub>4</sub>)<sub>2</sub> and NH<sub>4</sub>HSO<sub>4</sub> formed by the reaction between NH<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> (ref. 17), or by NH<sub>3</sub>-enhanced oxidation of SO<sub>2</sub> (ref. 18). The occasional presence of NH<sub>4</sub>HCO<sub>3</sub> in throughfall and stemflow, however, indicates that direct absorption of NH<sub>3</sub> on vegetation surfaces followed by partial neutralization by carbon dioxide may precede the formation of ammonium sulphate. Similarly, dilute sulphuric acid formed from SO<sub>2</sub> deposited on leaf surfaces<sup>5</sup> may trap gaseous NH<sub>3</sub>.

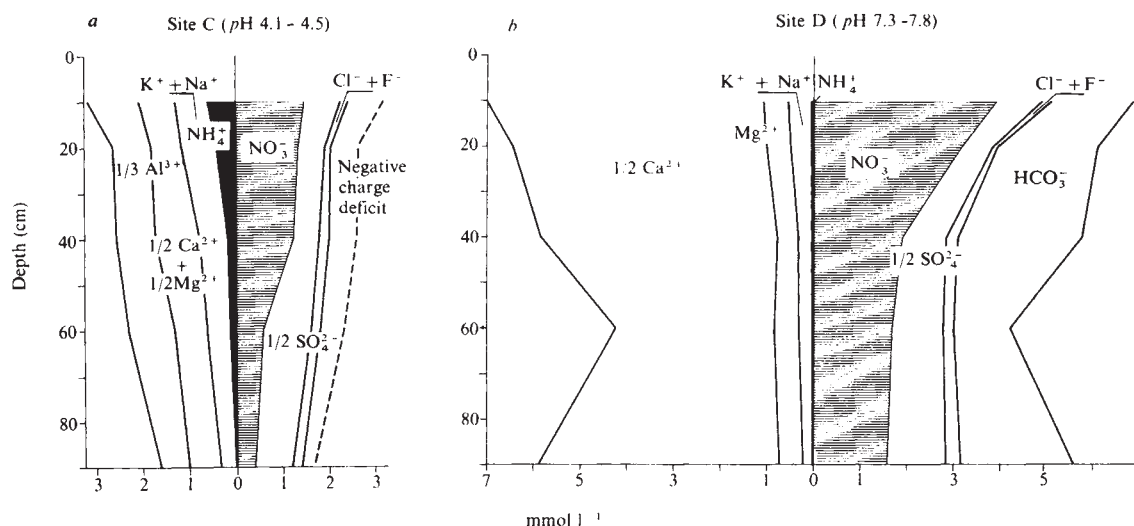
The fate of ammonium sulphate on entering the soil is illustrated by the ionic composition of the soil solution at location 1. The soil solution was sampled monthly by suction from Soil Moisture Equipment porous cup lysimeters at 10, 20, 40, 60 and 90 cm depth (with duplicate collectors at 10, 40 and 90 cm) at each of the four sites. The sampled points were ~3–4 m from the nearest tree. In addition to chemical analyses of throughfall and stemflow, soil solutions were assayed for total dissolved aluminium (colorimetrically with pyrocatechol violet after destruction of organic matter by aqua regia, and partly by atomic absorption spectrophotometry)<sup>6</sup>. Typical profiles of the soil solution composition for sites with acidic and calcareous soils are shown in Fig. 2. In the calcareous soil, NH<sub>4</sub><sup>+</sup> is virtually absent and NO<sub>3</sub><sup>-</sup> is the dominant anion in the surface soil. The acidic soil contains more dissolved NH<sub>4</sub><sup>+</sup> and less NO<sub>3</sub><sup>-</sup> than the calcareous soil, but NO<sub>3</sub><sup>-</sup> still predominates. These observations indicate the essentially complete nitrification of atmospheric ammonium sulphate in the calcareous soil and its partial

nitrification in the acidic soil. A mixture of nitric and sulphuric acid is formed during nitrification:



In the calcareous soil the acids dissolve calcium carbonate, leading to a predominance of dissolved calcium nitrate. In the acid soils, aluminium nitrate is formed by dissolution of minerals containing aluminium. The importance of nitrification in soil acidification is shown by the significant negative correlation ( $r = -0.6$ ) between pH (range 2.9–5.5) and dissolved NO<sub>3</sub><sup>-</sup> (range 0.56–5.5 mmol l<sup>-1</sup>) compared with that of -0.23 between pH and SO<sub>4</sub><sup>2-</sup> (range 0.51–2.31 mmol l<sup>-1</sup>) in 56 soil solution samples at 20–40 cm depth in the non-calcareous soils studied at location 1.

Not all the NO<sub>3</sub><sup>-</sup> in the soil solution necessarily came from throughfall: mineralization and nitrification of organic nitrogen from decomposing plant remains also contribute to dissolved NO<sub>3</sub><sup>-</sup>, whereas uptake of NO<sub>3</sub><sup>-</sup> by plants has the opposite effect. Also, direct adsorption and assimilation of agricultural NH<sub>3</sub> (ref. 19) would reduce NO<sub>3</sub><sup>-</sup> uptake by plant roots, thereby increasing NO<sub>3</sub><sup>-</sup> concentrations in the soil solution. In undisturbed forest ecosystems in less polluted areas, ionic uptake by the vegetation maintains much lower levels of NO<sub>3</sub><sup>-</sup> in soil solutions and drainage water<sup>12,20–23</sup>. Between March and October 1981, the pH of the soil solution decreased gradually, reaching values as low as 2.8, while nitrate concentrations rose concomitantly. Between November 1981 and March 1982 pH values increased again to 3.5–4 and nitrate concentrations were



**Fig. 2** Composition of the soil solution at five different depths (10, 20, 40, 60 and 90 cm) from a non-calcareous soil (a; site C) and a calcareous soil (b; site D) at location 1, sampled 12 May 1981. Concentrations are shown cumulatively for anions and cations separately. The negative charge deficit at C may be due to organic acids.

reduced. The presumably seasonal acidification is attributed to a continuous supply and nitrification of  $\text{NH}_4^+$  from throughfall and decomposing organic matter, with little or no leaching (because evapotranspiration generally exceeded precipitation) in summer, and limited  $\text{NO}_3^-$  uptake by plants.

If all ammonium is nitrified, the estimated annual acid inputs from throughfall and stemflow alone at locations 1 and 2 are, respectively, 713 and 723  $\text{kmol km}^{-2}$ , equivalent to 800 mm annual precipitation of pH 3.0. Actual inputs are probably higher, because part of the gaseous  $\text{NH}_3$  trapped and assimilated by the vegetation is leached from the soil after mineralization. Even so, the calculated acid inputs are considerably higher than those reported for other areas having serious acid rain deposition: 280 and 460  $\text{kmol km}^{-2} \text{yr}^{-1}$  in beech and spruce forests in FRG<sup>5</sup>, 220  $\text{kmol km}^{-2} \text{yr}^{-1}$  in pine forests in Birkeness, Norway<sup>24</sup>, and 136  $\text{kmol km}^{-2} \text{yr}^{-1}$  at Hubbard Brook, USA<sup>25</sup>. The presence of ammonia on leaves may enhance deposition and oxidation of  $\text{SO}_2$  (ref. 18) and may further contribute to the high deposition rates reported here. Similar effects probably occur in other regions with intensive animal husbandry, for example, large parts of Western Europe. It may be locally important in the USA, for example, in areas with cattle feedlots<sup>26</sup>.

The processes discussed above involve high removal rates of anthropogenic N and S from the atmosphere by forests, and therefore should profoundly influence the quality of air, soil and groundwater, and may affect the vegetation. Trees and herbaceous plants (mainly *Pteridium aquilinum*, *Maianthemum bifolium*, *Lonicera periclymenum* and *Rubus fruticosus*) appear to grow normally, however, in spite of the extremely low pH observed in the non-calcareous soils at location 1. Although the inputs of nitrogen and acid have obviously increased over the past few decades, floral composition of the herbaceous layer at location 1 has changed little in the past 25 yr (G. Minderman and B. F. M. Wijlens, personal communication).

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## Skylight polarization patterns at dusk influence migratory orientation in birds

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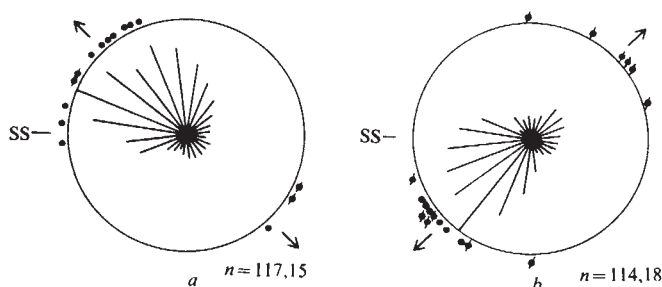
Over 100 animal species are known to be able to perceive the plane of polarization of linearly polarized light; most of these are arthropods but several molluscs and vertebrates, including man, share this ability<sup>1,2</sup>. At least five aquatic vertebrates (three fish, a salamander and a larval frog) are capable of oriented movement based on the E-vector of linearly polarized light<sup>3–9</sup>. Pigeons (*Columba livia*) have been conditioned to discriminate between rotating and stationary polarized light and between widely separated stationary E-vectors<sup>10,11</sup>, but the behavioural significance of this ability remains unknown. I report here that the migratory orientation of the white-throated sparrow (*Zonotrichia albicollis*), a nocturnal migrant, is affected by manipulations of the axis of skylight polarization. These data provide the first evidence that polarized light may be a relevant cue in migratory orientation.

Several sources of directional information are known to be involved in migratory orientation in birds: Sun, star and magnetic compasses, and wind direction. Considerable redundancy characterizes the system and hierarchical relationships exist for at least some of the cues<sup>12</sup>. Many birds are predominantly nocturnal migrants, but recent evidence shows that information from the Sun late in the afternoon influences migratory orientation during the following night<sup>13–15</sup>. Whether the Sun is used directly (for example, as a fixed reference point or time-compensated compass), or indirectly via polarization patterns is unknown; at least a few nocturnal migrants possess a time-compensated Sun compass<sup>16,17</sup>.

Light from a clear blue sky is linearly polarized. The degree of polarization is often high, typically 70–80% near the zenith at dawn or dusk<sup>18–20</sup>. In addition, both the degree of polarization and the orientation of the E-vector axis are related directly to the position of the Sun. Polarization is maximal at 90° away from the Sun and the E-vector is everywhere perpendicular to the plane containing the Sun, the point in the sky being observed, and the observer. Thus sky polarization can be used as an extension of Sun compass orientation as shown by Frisch for honeybees (*Apis mellifera*)<sup>21</sup>. The fixed relationship between E-vector direction at a given point in the sky and Sun azimuth could also be used as a fixed point of reference at a given time of day (for example, the period between sunset and darkness for a nocturnal migrant bird). In addition, navigational information is available in the dynamic patterns of skylight polarization that result from the Earth's rotation, but no animal is yet known to use this<sup>22</sup>.

Birds frequently initiate nocturnal migration during the period between sunset and darkness and in orientation cages they characteristically show oriented hopping during twilight when in the migratory state. The present tests were performed under clear skies between the time the Sun's disk disappeared below the local horizon and the time the first bright stars became visible (~50 min later). Each bird was placed in a circular orientation cage in which hopping was recorded as ink footprints on a blotter paper funnel<sup>23</sup>. The top of each cage was completely covered with a linear dichroic polarizer (Plexiglass laminated HN-38; Polaroid) that allowed leakage of no more than 0.005% unpolarized light. Tests were performed on a hilltop in Berne, Albany County, New York, between 22 May and 5 June 1981, using birds captured locally during migration. Birds could see the sky nearly to the horizon, but no objects on the ground were visible.





**Fig. 1** The orientation of white-throated sparrows tested outdoors at dusk was parallel to the E-vector of imposed plane-polarized light. *a*, Polarizer E-vector 45° clockwise from the azimuth of sunset. *b*, Polarizer E-vector 45° anticlockwise from the azimuth of sunset. Two distributions are plotted. The vectors inside the circles show the pooled hopping activity of the sparrows tested in each condition; they are plotted such that the unit radius equals the greatest number of units of activity in any 15° sector (given as the first number at the lower right of each circle). The dots on the periphery of each circle represent the mean directions of the pooled activity of each bird tested; dots with bars represent the two modes for birds that showed bimodal activity. Each of these points (second number at lower right) was used to compute the resultant vectors. The distributions of pooled activity and of means from both groups were significantly oriented (Rayleigh tests,  $P = 0.0001$ , both conditions; Hotelling  $T^2$ -tests,  $P = 0.001$ , both conditions<sup>24</sup>). Mean vectors of pooled activity: condition 1(*a*), 2.1° clockwise from the northwestward end of E-vector,  $r = 0.379$ ; condition 2(*b*), 14.7° clockwise from the southwestward end of E-vector,  $r = 0.360$ . SS, sunset direction.  $\leftarrow$ ,  $\rightarrow$ , E-vector.

On each night, 10 birds drawn from a pool of 19 individuals were randomly assigned to one of two groups tested simultaneously in one of two conditions: (1) polarizer E-vector oriented 45° clockwise from the azimuth of sunset; (2) polarizer E-vector oriented 45° counterclockwise from the azimuth of sunset. The two groups were thus exposed to skies of identical colour and light intensity patterns, but which had E-vectors that differed from each other by 90° and from the natural zenith sky E-vector by  $\pm 45^\circ$ . The polarized light to which the birds were exposed differed in other ways from natural skylight polarization: (1) viewed through the polarizers, light from all parts of the sky was almost 100% polarized; (2) the E-vector was the same in all parts of the sky; (3) short wavelengths (400  $\mu\text{m}$ ) were drastically attenuated.

Nearly all the birds showed clearly oriented hopping during each test (the average vector length for all tests was  $\bar{r} = 0.653$ , s.d. = 0.241). The pooled activity of the birds in each condition and the mean direction computed from the hopping of each bird pooled over all of its tests are plotted in Fig. 1. The distributions of pooled activity and of means for both groups were oriented parallel to the E-vector of polarization to which each was exposed. Thus in terms of geographical directions the two groups of birds hopped orthogonally to each other. The two distributions (Fig. 1*a, b*) are significantly different from each other ( $\chi^2 = 126.4$ , d.f. = 3,  $P < 0.0001$ ). Thus, the apparent orientation of the axis of skylight polarization had a major effect on the directional hopping of the sparrows.

During spring and autumn migration in north temperate latitudes, the natural twilight zenith E-vector provides an approximately north-south axis, corresponding to the main flow of bird migration. However, polarization patterns of the type presented to the birds in these experiments do not provide unambiguous compass directions: the two 'ends' of the E-vector cannot be distinguished. However, in both conditions the birds showed a strong bias towards the end of the E-vector axis nearer to the sunset. In condition (1) (Fig. 1*a*) this resulted in seasonally appropriate northwestward orientation, but in condition (2) (Fig. 1*b*) the birds oriented to the south-west or west. The natural skylight pattern provides numerous cues available to resolve this ambiguity: colour or light intensity patterns, the degree of polarization, or the angular change in the E-vector

over a small patch of sky<sup>20</sup>. In the artificial patterns viewed by the birds only the colour and intensity differences remained. In neither group was there a marked tendency to hop directly towards the brightest part of the sky (directly towards sunset), but it seems probable that intensity and/or colour patterns were involved in the departure of the orientation from true bidirectionality. Had the birds been using either the Sun compass or magnetic compass, both groups would be expected to orientate towards the northward end of their respective E-vectors, that is, approximately NW for group *a* and NE for group *b* of Fig. 1. In fact, this is not the case: the spring orientation of white-throated sparrows when tested under natural skies at this locality is consistently towards NW-NNW.

Previous studies of the role of solar cues in the orientation of nocturnal migrants have shown an effect of exposure to clear skies at sunset on behaviour that probably occurred after stars became visible<sup>13-15</sup>. The evidence that polarized light has behavioural relevance to the orientation of a nocturnally migratory bird, makes it uncertain that the Sun itself is involved in nocturnal migratory orientation. It seems probable that polarization patterns provide yet another source of information in what is known to be a highly redundant system of compass cues. However, these results open the possibility that the more complex and useful navigational information (for example, latitude, local Sun time) contained in the changing patterns of skylight polarization with time<sup>22</sup> could be used by birds.

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## Prolactin and parental behaviour in a male New World primate

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In some mammals, both sexes exhibit a high degree of parental behaviour. This is the case in many primates which form pair bonds, such as marmosets, tamarins, owl monkeys and siamangs<sup>1-4</sup>. It is not known if any endocrine changes occur in male mammals which show parental behaviour. We report here that in male common marmosets carrying their twin offspring,

plasma prolactin levels are five times higher, on average, than in males without infants. There are, however, no consistent differences in levels of plasma testosterone. Increases in prolactin are most pronounced during periods when males (rather than other group members) are carrying their offspring. This suggests that physical contact may be important in producing the effects. Although prolactin has been implicated in the control of maternal behaviour in avian and mammalian species<sup>5-8</sup> this is the first demonstration that prolactin is elevated during parental behaviour in a male mammal.

Fifteen captive family groups of common marmosets (*Callithrix jacchus*) were studied in order to obtain blood samples from five males paired with non-pregnant females, five males paired with pregnant females and five males paired with females and their twin offspring aged 10–30 days. Older offspring (juveniles or subadults) were present in some groups but only five of them contained young infants which were transported by their parents. Blood samples were collected daily and up to 10 samples were taken from each male, using a restraint procedure to which the animals had been habituated<sup>9</sup>. Males readily habituated to the removal of infants which were passed, using the gloved hand, directly to their mothers. It was possible to obtain blood samples within 60–120 s of handling males. Groups containing infants were observed immediately before blood sample collections. Only those males which carried offspring on at least 60% of these occasions were included in the study. All samples were obtained during 10.00–13.00 h to minimize circadian variations in plasma prolactin and testosterone, which were measured by radioimmunoassay<sup>10,11</sup>.

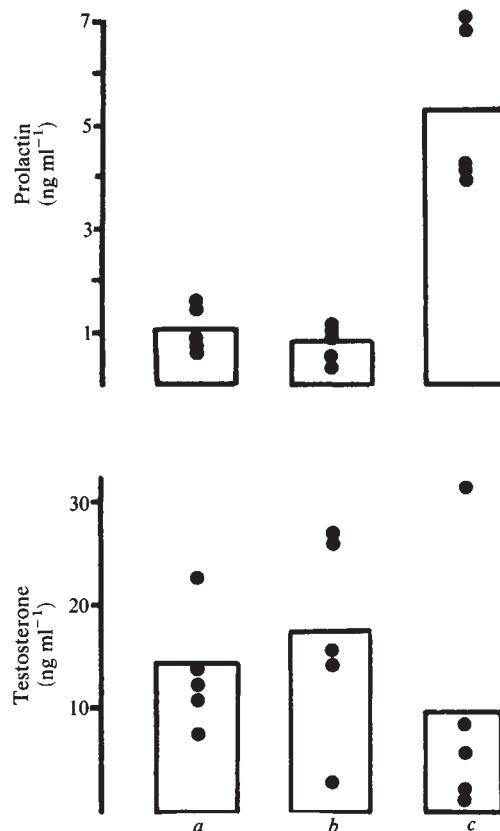
Results are shown in Fig. 1; levels of prolactin or testosterone are expressed as means for each male. Analysis of variance followed by the Scheffé test<sup>12</sup> was carried out on these mean values (five for each of the three groups). Prolactin levels (mean  $\pm$  s.e.m.) were significantly higher ( $P < 0.001$ ) in males with young infants ( $5.3 \pm 0.5$  ng ml<sup>-1</sup>) than in males with no infant offspring caged with either pregnant females ( $0.84 \pm 0.07$  ng ml<sup>-1</sup>) or non-pregnant partners ( $1.08 \pm 0.08$  ng ml<sup>-1</sup>). No such differences were found for testosterone levels. Nor did prolactin levels differ significantly in males without infants irrespective of whether their females were pregnant or not ( $P > 0.05$ ). Mean levels of plasma prolactin in the five males with infants ranged over 4.0–7.0 ng ml<sup>-1</sup> and maximum concentrations measured in each animal were 6.2, 11.5, 14.5, 15.5 and 18.0 ng ml<sup>-1</sup>. By contrast, in the 10 males without infants, plasma prolactin never exceeded 2.0 ng ml<sup>-1</sup>. Prolactin levels in males with infants were comparable to those measured in 40 blood samples from 13 non-lactating females (non-pregnant:  $0.5$ – $26.4$  ng ml<sup>-1</sup>,  $\bar{x}$  3.9; pregnant:  $1.5$ – $15.8$  ng ml<sup>-1</sup>,  $\bar{x}$  6.3). As expected, prolactin levels were much higher in four lactating females ( $7.5$ – $78.5$  ng ml<sup>-1</sup>,  $\bar{x}$  30.7 for 20 determinations). The ranges are similar to those in ref. 10, but mean levels are lower, perhaps because less samples were measured and prolactin levels vary considerably depending on suckling activity and the stage of lactation considered (A. S. McNeilly, personal communication).

Testosterone levels were low in some males with infants but one animal had the highest concentrations recorded in any of the males ( $32.4 \pm 8.1$  ng ml<sup>-1</sup>, Fig. 1). A Spearman rank correlation coefficient revealed no consistent relationship between mean levels of testosterone and prolactin in the 15 males ( $r.s. = -0.20$ , not significant). As up to 10 samples had been obtained from each animal, possible correlations between testosterone and prolactin levels were examined in each individual, but no significant relationship was found ( $r.s. < 0.42$ ,  $P > 0.05$  in all cases). Thus the increases in plasma prolactin which occurred in males with infants were not necessarily associated with decreases in circulating testosterone. Chronic hyperprolactinaemia may be associated with lowered testosterone levels in human males<sup>13</sup>, or male rhesus monkeys treated with dopamine antagonists<sup>14</sup> although this is not invariably the case.

Prolactin concentrations were higher on days when males were carrying infants immediately before blood sampling ( $n =$

37,  $6.9 \pm 0.6$  ng ml<sup>-1</sup>) than on days when infants were 'off' their male parent ( $n = 13$ ,  $2.3 \pm 0.3$  ng ml<sup>-1</sup>,  $P < 0.001$ , two-tailed,  $t$ -test). This suggests that increases in plasma prolactin are associated with periods of physical contact between the male and its offspring. In preliminary experiments plasma prolactin was measured in one male which failed to carry its twin infants. Hormone levels were very low in this case ( $n = 9$ , mean  $0.6 \pm 0.23$  ng ml<sup>-1</sup>). It is also appropriate to mention that female marmosets with twins show higher levels of circulating prolactin than females with a single offspring<sup>10</sup>.

We have verified that the procedures used to collect blood samples do not have short-term effects on plasma prolactin levels and that physical contact in another behavioural context (sexual behaviour) does not influence prolactin secretion in the



**Fig. 1** Plasma levels of prolactin and testosterone in male marmosets in captive family groups containing: *a*, non-pregnant females; *b*, pregnant females; or *c*, females with infants aged 10–30 days. Histograms each show mean levels for five males. Each point represents mean plasma levels for 10 samples from a single male, except for two of the males caged with pregnant females. Six and seven samples respectively were collected from these animals before their female partners gave birth. Overall analysis of variance revealed a statistically significant difference in prolactin levels (but not in testosterone levels) between the three groups ( $F = 34.63$ , d.f. 2, 14;  $P < 0.002$ , two-tailed test). Further analysis using the Scheffé test, showed that males in group *c* with young infants had significantly higher prolactin levels ( $P < 0.001$ ) than males in groups *a* and *b*. Radioimmunoassays of prolactin used a guinea pig, anti-human antiserum which has been validated for measurement of marmoset prolactin by McNeilly *et al.*<sup>10</sup>. The <sup>125</sup>I-labelled ovine prolactin used as tracer and ovine prolactin standard were also the same as in ref. 10. Inter- and intra-assay coefficients of variation were 11.2% ( $n = 8$ ) and 5.1% ( $n = 10$ ) respectively. Testosterone was measured using an ovine antiserum supplied by the World Health Organization, which has 14% cross-reactivity with 5 $\alpha$ -dihydrotestosterone (DHT) but negligible cross-reaction with other steroids. Plasma levels of testosterone measured before and after Celite column chromatography to remove DHT do not show significant differences ( $t = 0.10$ , d.f. 3;  $P > 0.9$ ). Hence it is unlikely that cross-reaction with DHT interfered with the measurement of testosterone. Inter- and intra-assay coefficients of variation were 12.5% ( $n = 8$ ) and 6.5% ( $n = 8$ ), respectively.

male marmoset. Blood samples were obtained from five males before (5 min), and after (5, 15, 25 and 35 min) a 10-min behaviour test during which mating occurred. Prolactin levels were low initially ( $1.2 \pm 0.3 \text{ ng ml}^{-1}$ ) and a two-way analysis of variance showed that no significant differences occurred either between sampling times ( $F = 1.35$ , d.f. 4, 16; not significant) or between subjects ( $F = 2.67$ , d.f. 4, 16; not significant). A second experiment using the same subjects and sampling times (but without a behaviour test) confirmed that no changes in prolactin levels occurred in the short term ( $F = 0.84$ , d.f. 4, 16; not significant). Long-term effects also seem unlikely as, in 13 males from which 10 daily blood samples were obtained, prolactin levels in the first and last samples did not differ significantly ( $P > 0.05$ , paired  $t$ -test). The possibility that very rapid increases in prolactin levels might occur in males with infants during the 60–120 s required to obtain blood samples was examined by drawing samples consecutively from five males at 60, 120 and 180 s after removal of their twin offspring. Prolactin levels at these times averaged 6.65, 6.13 and  $7.0 \text{ ng ml}^{-1}$  respectively ( $F = 0.20$ , d.f. 2, 8; not significant), indicating that

increases in circulating prolactin do not occur under these circumstances.

It is well known that suckling and other stimuli from the offspring enhance the secretion of prolactin in female mammals, although the involvement of this hormone in the control of maternal behaviour remains controversial<sup>6–8</sup>. The finding that prolactin levels rise in male monkeys which exhibit parental behaviour is surprising and is the first demonstration of such an endocrine change in any mammalian species. As infant marmosets cling tightly to the male it is possible that tactile stimulation of the nipples or occasional suckling attempts might affect prolactin levels. The causes of the phenomenon and its possible behavioural significance are being examined.

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## Mechanisms of interpolation in human spatial vision

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Recently, interest has revived in the classical problems of vernier and stereoscopic acuity. The precision with which observers can align two bars in a vernier task is as high as 2 sec arc, which is much finer than the grain of the retinal receptor mosaic, where the cones are separated by 20–30 sec arc even at their most densely packed. Thus, it seems that the visual system may be able to interpolate between the discrete samples provided by the retinal mosaic; recently, several possible mechanisms have been proposed<sup>1,2</sup>. Mathematically there is no mystery about interpolation. The optical system of the eye acts as a low-pass filter removing all frequencies above  $\sim 60 \text{ cycles deg}^{-1}$ , so it follows from the sampling theorem that the retinal image can be fully represented by sampling at a frequency of 120 cycles  $\text{deg}^{-1}$ , which is approximately that of the receptor mosaic. In principle, therefore, discrete sampling by the retinal mosaic does not remove information from the image. Indeed, the low-pass filtering action of the eye implies that acuity would not be lost, even if the signal itself were divided into discrete samples, before observation. We report here that human observers can locate the spatial position of a periodic visual pattern with a precision as high as 5–10 sec arc, even though the pattern is coarsely sampled at an interval over 10 times that amount. This suggests that the human visual system can construct a surprisingly accurate representation of a pattern from discrete samples, despite the samples being sufficiently widely spaced to be visually resolved.

Our method of spatial sampling is shown in Fig. 1 (see also ref. 3). We used an oscilloscope under computer control to construct two ribbons of light ( $12 \text{ min} \times 2 \text{ deg arc}$ ) along each of which luminance was modulated sinusoidally. To avoid discontinuities at the ribbon edges, the contrast of this modulation

was itself multiplied by a gaussian, so that only the central 3–5 cycles were actually visible. The observer's task was to decide whether the luminance profile of the top stimulus was to the left or right of that of the lower stimulus. Thresholds for detecting a spatial offset were determined by APE, a modified method of constant stimuli<sup>4</sup>, and are defined as the 83% point of detection.

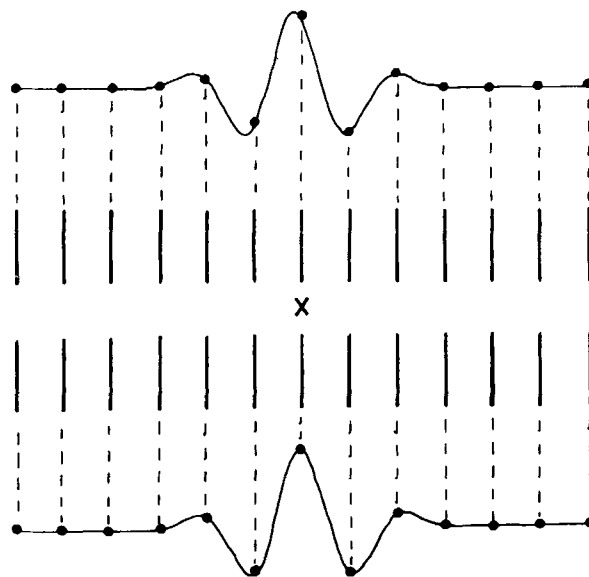
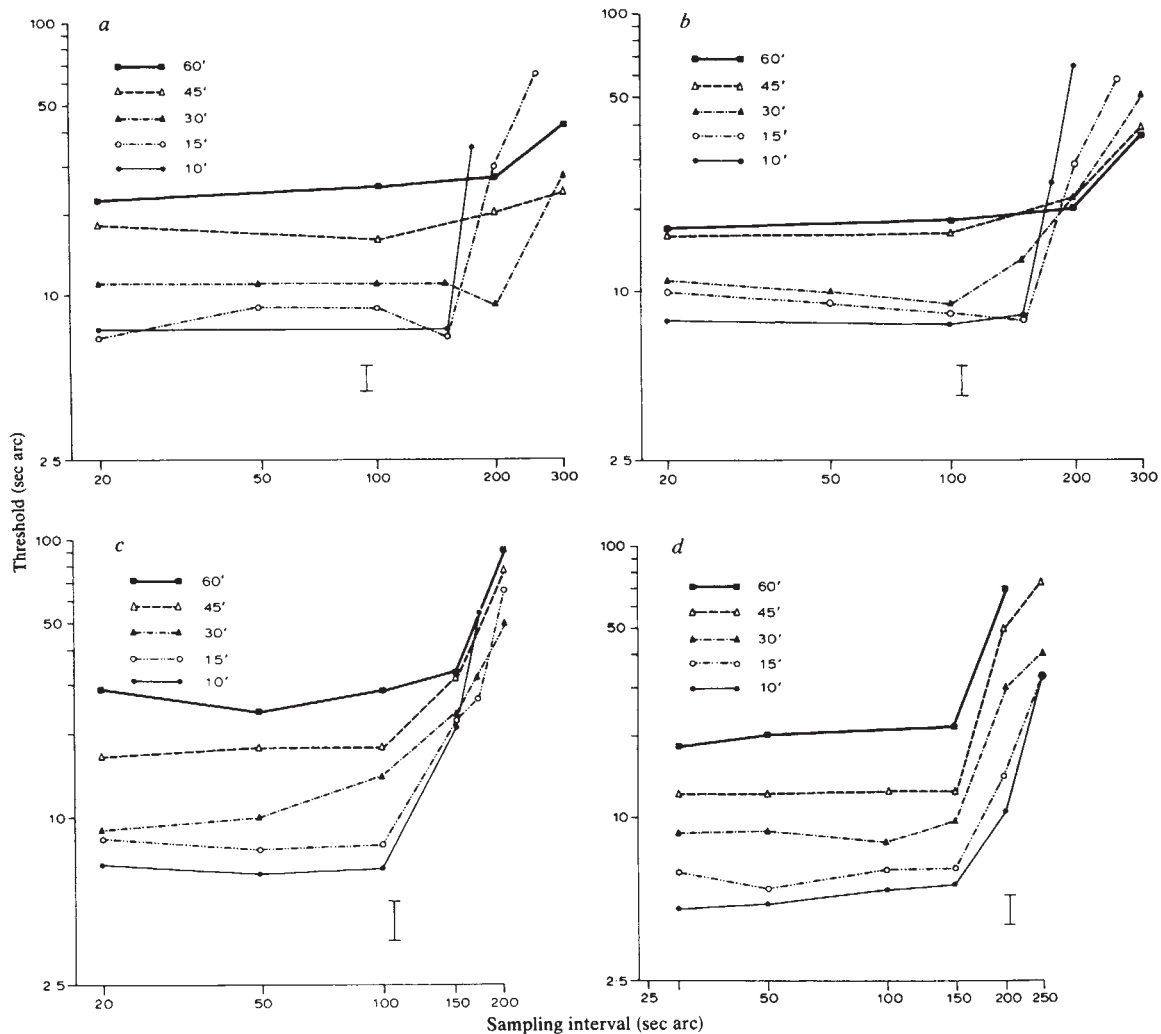


Fig. 1 Observers judged the alignment of the two luminance profiles shown at the top and bottom of the figure. The profiles were identical except that the top one was spatially shifted to one side (in this case the left). In the stimulus actually seen by the subject, each of the luminance profiles was displayed at discrete points along a horizontal ribbon; these sample points are represented by dots on the profiles, and by vertical lines in the ribbon beneath. Each vertical line has the luminance corresponding to the dot on the profile. Note that the samples have the same position in the two ribbons: only the profiles that they represent are shifted. It is found that observers can reconstruct accurately the spatial position of the profiles being represented, despite the discreteness of the sampling.





**Fig. 2** *a, b*, Results of the vernier alignment task described in Fig. 1 and the test, for two different observers, C.C. and R.J.W., respectively. The threshold spatial offset for 83% correct detection (ordinate) is shown as a function of the sampling interval (abscissa), the parameter of the curves being the spatial period in min arc of the luminance profile. *c, d*, Results of a test of stereoscopic acuity, analogous to that of vernier acuity in *a, b*. The two observers were M.J.M. (*c*) and R.J.W. (*d*). For further explanation see text.

So far this is a conventional vernier alignment task, except that the two stimuli are sinusoidal luminance profiles rather than bars. However, we then displayed the original stimulus only at discrete points along the ribbon, as shown in Fig. 1. Each stimulus was now represented by a series of discrete vertical bars of the exact luminance of the reference profile at that position in space. The sampling points were at identical horizontal positions in the two stimuli: only their luminance varied to provide a cue for response. In a variation of this procedure, we introduced a random spatial shift in the position of sampling, so that the samples in the two ribbons were no longer aligned, but were still uninformative *per se* about the location of the profiles that they represented. The observer's task was to judge the alignment of the luminance profiles, not the samples themselves.

Figure 2*a, b* shows the results for the vernier acuity test for two subjects and for various spatial frequencies. In all cases the contrast of the grating was 60%. Our basic finding is that acuities in the range 5–20 sec arc are not degraded by discrete sampling of the stimulus up to an inter-sample distance of 150–200 sec arc. For the cases where the individual samples were not resolved this finding is not particularly interesting, as interpolation can be thought of as being carried out by the optics of the eye. But the samples become clearly resolved at spacings of 60–90 sec arc, and the data show that there was no drop in acuity at that point. Thus, the profile represented by the samples could be discerned with undiminished spatial accuracy, even when the samples themselves were visually resolved.

We found the same effect with stereoscopic acuity, and here our findings give some clue to the functional advantage of long-range interpolation. The stereo task was carried out with a dichoptic display in which each eye saw a stimulus configuration similar to that in Fig. 1. An effective disparity was produced in the top ribbon by moving its luminance profile in opposite directions in the two eyes. As in the vernier task, the spatial position of the samples themselves was constant, and the observer had to judge whether the top profile was nearer or further away than the bottom one. Figure 2*c, d* shows that this task could be carried out with high precision, up to sampling distances of ~150 sec arc. The phenomenal appearance of the stimuli was curious: when the samples were sufficiently widely spaced to be resolved, but still within the interpolation range, we saw the samples at one depth plane (which did not differ



**Fig. 3** This figure illustrates the convolution between a discretely sampled signal and an interpolation function consisting of the difference of two gaussians (DOG). The original signal consisted of a 6 cycle  $\text{deg}^{-1}$  sine wave multiplied by a gaussian contrast function (s.d. of 5 min arc) and sampled at intervals of 108 sec arc. The DOG has excitatory and inhibitory s.d.s of 75 and 120 sec arc, respectively, and is the 'N' channel, the smallest proposed by Wilson and Bergen<sup>5</sup>. Zero-crossings occur where the function in the figure crosses the horizontal line.

between the two ribbons) and the profile that they represented at another. This suggests that interpolation could be useful in constructing the appearance and depth of objects that are partially occluded by intervening objects.

We now consider the question of a mechanism for long-range interpolation beyond the spacing of the retinal mosaic. Barlow<sup>2</sup> suggests that the densely packed cell layer IVC of the visual cortex is the site of interpolation, which could be carried out by a series of graded neural input connections. Certainly neural divergence would be required for interpolation, the essence of which is to represent the signal at a set of positions much more finely spaced than the original sample points.

Mathematically, interpolation can be carried out by convolving the sampled function with an appropriate frequency-limited function. The latter should be chosen to contain these frequencies present in the original stimulus, and not their higher harmonics introduced by sampling. If the bandwidth of the interpolator is too broad, these higher harmonics will be included, thus interfering with the signal and reducing the accuracy with which it can be represented. We suggest that this is exactly the case for the human visual system, as illustrated by our data. Irrespective of the frequency content of the signal, the minimum sampling frequency for high acuity is  $\sim 25$  cycles  $\text{deg}^{-1}$ , suggesting that the transmission system has too broad a bandwidth to interpolate optimally. For example, we suggest that the simple difference of Gaussians (DOG) model of receptive field organization<sup>5</sup> is adequate to explain the precision of interpolation found in our psychophysical experiments, and to explain the failure of interpolation for larger sampling intervals. To illustrate this, Fig. 3 shows the convolution of a sinusoidal luminance profile sampled at intervals of 108 sec arc, and a DOG function with s.d.s of 75 and 120 sec arc for the excitatory and inhibitory components, respectively (the 'N' channel of Wilson and Bergen<sup>5</sup>). It is evident that interpolation is just beginning to fail; this is because the bandwidth of the channel is too broad. However, several features of the original signal which would permit accurate localization are preserved. In particular, if we adopt the Marr and Hildreth model of edge detection<sup>6</sup>, which would use the zero-crossings in Fig. 3, we can see why the precision of spatial location judgments can survive sampling at intervals considerably wider than that of the retinal mosaic or the line spread function of the eye.

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## Potent new inhibitors of human renin

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The renal acid protease renin selectively cleaves its plasma substrate angiotensinogen to release the decapeptide angiotensin I, which in turn is cleaved by a carboxydipeptidase converting enzyme to yield the pressor octapeptide angiotensin II<sup>1</sup>. It is generally accepted that the renin-angiotensin system has a physiological role in blood pressure and electrolyte homeostasis<sup>2</sup>, and that abnormalities of the system contribute to certain forms of hypertension<sup>3</sup>. We previously showed that reduction of the Leu<sup>10</sup>-Leu<sup>11</sup> scissile peptide bond in the (6–13) octapeptide sequence 2 of equine angiotensinogen produced

potent and selective inhibitors of canine plasma renin<sup>4</sup>. We have now used the same approach to modify the recently elucidated N-terminal sequence<sup>6</sup> of human angiotensinogen and report here the production of highly active and species-specific *in vitro* inhibitors of endogenous human renin cleaving renin substrate in human plasma. Whereas the (6–13) octapeptide H-112 of human angiotensinogen is only a weak inhibitor of human plasma renin ( $\text{IC}_{50} = 313 \mu\text{M}$ ), the analogue H-113, in which the scissile Leu-Val bond has been reduced, has  $\text{IC}_{50} = 0.19 \mu\text{M}$ . Extension of H-113 with Pro at the N-terminus and with Lys at the C-terminus<sup>7</sup>, giving the decapeptide derivative H-142, increases inhibitory potency further ( $\text{IC}_{50} = 10 \text{ nM}$ ). We believe that the high *in vitro* inhibitory potencies of H-113 and H-142 are due to the ability of the reduced moiety  $-\text{CH}_2-\text{NH}-$  to act as a non-hydrolysable analogue of the tetrahedral transition state formed during hydrolysis of the (10–11) peptide bond. H-113 and H-142 are strongly species specific, and are highly specific for renin among acid proteases: H-142 has no inhibitory effect *in vitro* on human cathepsin D or renal acid protease at a concentration of  $712 \mu\text{M}$ .

The N-terminal tetradecapeptide sequence 1 (Table 1) of equine angiotensinogen was elucidated by Skeggs *et al.*<sup>8</sup>. The smallest fragment of 1 still hydrolysed by renin at a significant rate is the (6–13) octapeptide 2, although this cleavage is slow enough to allow 2 to function as a weak ( $\text{IC}_{50} = 200 \mu\text{M}$ ) competitive inhibitor of the enzyme<sup>9</sup>. The minimum sequence still capable of acting as an inhibitor is the (10–13) tetrapeptide in the form of its methyl ester<sup>10,11</sup>. Substitution of amino acids in the angiotensinogen sequence has yielded more potent renin inhibitors<sup>12–14</sup>, some of which are also effective *in vivo*<sup>7</sup>.

We previously reported a novel approach to the development of renin inhibitors by chemical modification of the scissile Leu-Leu bond in a partial sequence of equine angiotensinogen<sup>4,5</sup>. Reduction of the Leu-Leu linkage to  $-\text{CH}_2\text{NH}-$  in the octapeptide 2 has yielded the analogue H-76 (Table 1) with  $\text{IC}_{50} = 0.03 \mu\text{M}$  against canine renin. Replacement of the His<sup>6</sup> residue in H-76 with D-His (compound H-77 in Table 1) has led to a further slight increase of inhibitory potency ( $\text{IC}_{50} = 0.02 \mu\text{M}$ ). Figure 1 shows the inhibition of dog renin by the equine octapeptide 2 and by the reduced analogues H-76 and H-77. Modification of the scissile bond has increased the inhibitory potency 10,000-fold. However, inhibitors based on the equine sequence show a significantly lower affinity to human than to dog renin. H-77 has  $\text{IC}_{50} = 1.0 \mu\text{M}$  against human renin, giving a human/dog potency ratio of 0.02. At the same time, the parent octapeptide 2 inhibits renin from both species equally (Table 1). Both H-76 and H-77 are active *in vivo* in lowering angiotensin I and II levels and blood pressure in the conscious dog<sup>5</sup>.

All work reported to date on substrate-related inhibitors of renin has been based on the equine octapeptide 2. There are sequence variations in the scissile regions of substrates from different species<sup>15</sup> which result in some degree of enzyme specificity: while human renin will cleave angiotensinogen from other mammals, only primate renin will hydrolyse human substrate. Although the equine octapeptide 2 binds equally to both the canine and human enzyme, the corresponding human

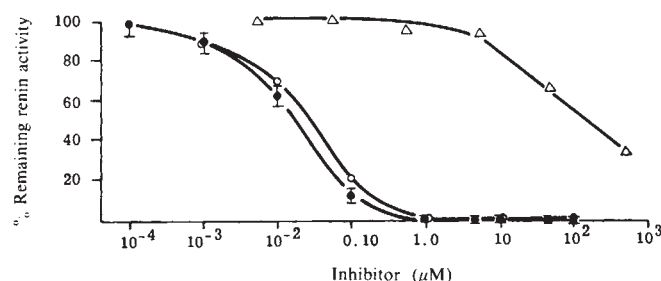


Fig. 1 Inhibition of renin in dog plasma by equine angiotensinogen (6–13) octapeptide 2 ( $\Delta$ ) and by its reduced analogues H-76 ( $\circ$ ) and H-77 ( $\bullet$ ).

**Table 1** Peptide sequences and inhibitory potencies of angiotensinogen analogues

| Code No. | Substrate sequence                                                        | IC <sub>50</sub> (μM) against renin from |       | Human/dog potency ratio |
|----------|---------------------------------------------------------------------------|------------------------------------------|-------|-------------------------|
|          |                                                                           | Human                                    | Dog   |                         |
|          | Renin<br>10 ↓ 11 12 13 14                                                 |                                          |       |                         |
| 1        | H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Tyr-Ser...                  | —                                        | —     | —                       |
| 2        | H-His-Pro-Phe-His-Leu-Leu-Val-Tyr-OH                                      | 200                                      | 200   | 1.0                     |
| H-76     | His-Pro-Phe-His-Leu- <sup>R</sup> -Leu-Val-Tyr-OH                         | 1.0                                      | 0.03  | 0.03                    |
| H-77     | H-D-His-Pro-Phe-His-Leu- <sup>R</sup> -Leu-Val-Tyr-OH                     | 1.0*                                     | 0.02† | 0.02                    |
| H-78     | H-His-Pro-Phe-His-Leu- <sup>R</sup> -Leu-Val-Tyr-OH<br>SO <sub>2</sub> Ph | 1.5                                      | —     | —                       |
| 3        | H-His-Pro-Phe-His-Phe-Phe-Val-Tyr-OH                                      | 31.0                                     | 500   | 16                      |
| H-110    | H-His-Pro-Phe-His-Phe- <sup>R</sup> -Phe-Val-Tyr-OH                       | 0.82                                     | 3.9   | 5                       |
| 4        | H-Pro-His-Pro-Phe-His-Phe- <sup>R</sup> -Phe-Val-Tyr-Lys-OH               | 5.9                                      | 140   | 24                      |
| H-108    | H-Pro-His-Pro-Phe-His-Phe- <sup>R</sup> -Phe-Val-Tyr-Lys-OH               | 0.04                                     | 0.6   | 15                      |
| 5        | H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His...                  | —                                        | —     | —                       |
| H-112    | H-His-Pro-Phe-His-Leu-Val-Ile-His-OH                                      | 313                                      | 1,000 | 3                       |
| H-113    | H-His-Pro-Phe-His-Leu- <sup>R</sup> -Val-Ile-His-OH                       | 0.19‡                                    | 155   | 800                     |
| H-142    | H-Pro-His-Pro-Phe-His-Leu- <sup>R</sup> -Val-Ile-His-Lys-OH               | 0.01§                                    | 10    | 1,000                   |

Peptides and their reduced analogues were synthesized by the solid-phase method according to Merrifield<sup>18</sup> on a 1% cross-linked chloromethyl resin support using *N*<sup>ε</sup>-Boc-protected amino acids. The side chain of His was protected with either *π*-2,4-dinitrophenyl<sup>19</sup> or *π*-benzyloxymethyl<sup>20</sup>, Tyr with 2,6-dichlorobenzyl<sup>21</sup> and Lys with 2-chlorobenzoyloxycarbonyl<sup>22</sup>. Coupling reactions were monitored with fluorescamine<sup>23</sup>. The reduced peptide bonds were introduced by the coupling, with dicyclohexylcarbodiimide/1-hydroxybenzotriazole<sup>24</sup>, of protected dipeptide analogues 6 Boc-NH-CH(R<sup>1</sup>)-CH<sub>2</sub>N(R<sup>2</sup>)-CH(R<sup>3</sup>)-CO<sub>2</sub>H where R<sup>1</sup> and R<sup>2</sup> represent the appropriate amino acid side chains, and R<sup>3</sup> the group protecting the secondary amine function formed during reduction of the peptide bond. The reduced analogue corresponding to Leu-Leu (6, R<sup>1</sup> = R<sup>2</sup> = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, R<sup>3</sup> = benzenesulphonyl) was prepared by reduction of Boc-Leu-Leu-OMe with sodium bis(2-methoxyethoxy)aluminium hydride, followed by protection of the secondary amine and re-oxidation of the C-terminal primary alcohol to carboxyl with alkaline permanganate<sup>25</sup>. The Leu-Val analogue (6, R<sup>1</sup> = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, R<sup>2</sup> = CH(CH<sub>3</sub>)<sub>2</sub>, R<sup>3</sup> = 3,4-dichlorobenzoyloxycarbonyl) and the Phe-Phe analogue (6, R<sup>1</sup> = R<sup>2</sup> = benzyl, R<sup>3</sup> = 3,4-dichlorobenzoyloxycarbonyl) were obtained by reductive alkylation of H<sub>2</sub>N-CH(R<sup>2</sup>)-CO<sub>2</sub>Bzl with the aldehyde Boc-NH-CH(R<sup>1</sup>)-CHO (ref. 26) using NaCNBH<sub>3</sub>, followed by removal of the benzyl ester group and introduction of the secondary amine protecting group R<sup>3</sup>. Structures of the protected reduced dipeptide analogues 6 were confirmed by NMR and IR spectra<sup>27</sup> and by elemental analyses. All compounds were purified by gel filtration and ion-exchange chromatography or HPLC, until found homogeneous by TLC, thin-layer electrophoresis and HPLC. Analysis after hydrolysis with 6M HCl at 110 °C for 24–48 h indicated the calculated ratios of amino acids. Residues corresponding to positions 10 and 11 were absent in the hydrolysates of the reduced peptide analogues. Instead, a single well defined new peak appeared in the region of the basic amino acids which was assigned to the reduced (10–11) dipeptide. Details of the syntheses either have been published<sup>27</sup> or will appear shortly (M.S., A.H., D.M.J., J.S. and B.A., in preparation). The IC<sub>50</sub> of the inhibitors was tested in a renin assay system<sup>28</sup> in which the rate of generation of angiotensin I by human plasma renin from human angiotensinogen at pH 7.0 was measured by an antibody-trapping radioimmunoassay<sup>28,29</sup>. A similar system was used with dog renin. The generation of angiotensin I in the presence of inhibitor was expressed as a percentage of that without inhibitor<sup>5</sup>. None of the inhibitors cross-reacted with antibody to angiotensin I at the highest concentrations used. 'R' represents a reduced peptide linkage, that is, -CH<sub>2</sub>NH- in place of -CONH-.

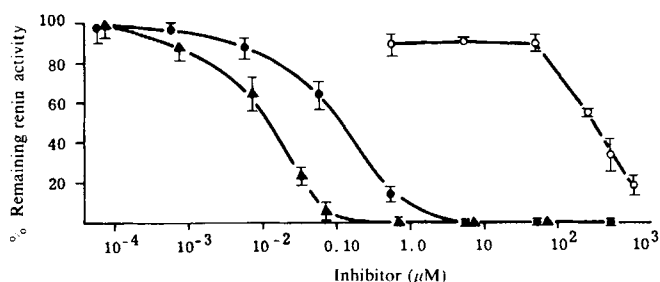
\* 1.0 ± 0.09 μM (mean ± s.e. of 8 experiments).

‡ 0.19 ± 0.06 μM (mean ± s.e. of 6 experiments).

† 0.02 ± 0.003 μM (mean ± s.e. of 6 experiments).

§ 0.01 ± 0.002 μM (mean ± s.e. of 4 experiments).

octapeptide H-112 shows a threefold preference for human renin (Table 1). Reduction of the Leu<sup>10</sup>-Val<sup>11</sup> scissile bond in the human octapeptide H-112 has produced the analogue H-113. The latter is 1,600 times more potent than its parent octapeptide in inhibiting human renin (IC<sub>50</sub> = 0.19 μM) while the inhibition of dog renin has increased only sixfold, giving a human/dog potency ratio of 800 (Table 1; Fig. 2).



**Fig. 2** Inhibition of renin in human plasma by human angiotensinogen (6–13) octapeptide H-112 (○) and by its reduced analogues H-113 (●) and H-142 (▲). The same pool of human plasma was used throughout. Inhibitors were dissolved in 0.01 M HCl and diluted with assay buffer. Generation of angiotensin I during a 30 min incubation at 37 °C was measured at pH 7.0 in the presence of inhibitor, or of solvent alone, using an antibody-trapping method<sup>28</sup>. The amount of angiotensin I produced in the presence of inhibitor is expressed as a percentage of that generated without inhibitor.

Prior to our work the most active substrate-derived inhibitor of renin was the Pro<sup>5</sup>,Phe<sup>10</sup>,Phe<sup>11</sup>,Lys<sup>14</sup>-analogue 4 of equine angiotensinogen (5–14) decapeptide, developed by Burton and co-workers<sup>7</sup>. In our test system, this compound is more effective as an inhibitor of human renin than either the equine octapeptide 2 or the Phe<sup>10</sup>,Phe<sup>11</sup> octapeptide 3 (ref. 13; Table 1). Reduction of the Phe<sup>10</sup>-Phe<sup>11</sup> bond in 3 has yielded the analogue H-110, which is 40 times more inhibitory against human renin and 130 times more inhibitory against dog renin than the parent peptide 3, resulting in a human/dog potency ratio of 5.

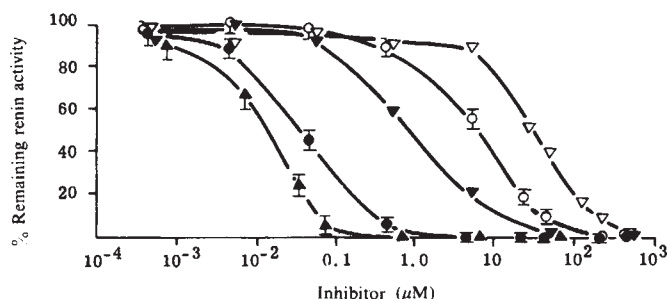
**Table 2** Recovery of renin inhibitors in human plasma and the effect of peptidase inhibitors on IC<sub>50</sub>

| Inhibitor | % Recovery* | IC <sub>50</sub> (μM) |       |
|-----------|-------------|-----------------------|-------|
|           |             | (1)                   | (2)   |
| H-112     | 61 ± 10.0   | 313 ± 59              | 240   |
| H-113     | 59 ± 4.0    | 0.19 ± 0.06           | 0.10  |
| 4         | 32 ± 4.0    | 5.9 ± 1.2             | 4.5   |
| H-108     | 26 ± 2.0    | 0.04 ± 0.007          | 0.035 |

The IC<sub>50</sub> of inhibitors was measured (1) without peptidase inhibitors<sup>28</sup> and (2) in the presence of EDTA (5 mM), *o*-phenanthroline (2 mM), benzamidine hydrochloride (5 mM) and Trasylol (50 μM). Mean ± s.e. shown if three or more experiments were carried out.

\* Inhibition of renin after 1 h preincubation in human plasma is expressed as a percentage of that without preincubation. Inhibitor concentration was chosen to give 50–99% initial inhibition.





**Fig. 3** Inhibition of renin in human plasma by the Phe<sup>10</sup>, Phe<sup>11</sup>-equine angiotensinogen (6–13) octapeptide 3 (▽), by its reduced analogue H-110 (▼), by the Pro<sup>5</sup>,Phe<sup>10</sup>,Phe<sup>11</sup>,Lys<sup>14</sup>-equine angiotensinogen (5–14) decapeptide 4 (○) and by its reduced analogue H-108 (●), compared with H-142 (▲).

When the Phe<sup>10</sup>-Phe<sup>11</sup> bond in the decapeptide 4 is reduced to give H-108, a 160-fold increase in the inhibition of human renin and a 230-fold increase in the inhibition of dog renin is observed (Fig. 3, Table 1).

A combination of the reduced peptide bond between positions 10 and 11, and the Pro<sup>5</sup>,Lys<sup>14</sup> substitutions proves synergistic when applied to human angiotensinogen, yielding the hitherto most potent inhibitor of human renin H-142 with IC<sub>50</sub> = 10 nM. This compound is 30,000 times more active than the human octapeptide H-112 from which it was derived and has a human/dog potency ratio of 1,000 (Table 1, Fig. 2).

To establish that the greater inhibitory potency of reduced analogues is not due to their increased stability in the assay system, we have tested the inhibition of renin by H-113 and H-108, and by their parent peptides H-112 and 4, in two conditions: (1) with and without a 1 h preincubation in human plasma and (2) with and without peptidase inhibitors. Our results indicate that there is no significant difference in stability between reduced analogues and their parent peptides (Table 2). Another possible explanation for the higher potency of reduced analogues is the introduction of a new basic centre at the site of cleavage which may form an ion pair with the catalytic carboxyl group of the enzyme. However, this possibility was discounted when it was found that H-78, a non-basic (but still tetrahedral) derivative of H-76, had a binding affinity comparable with that of the basic parent molecule.

We believe that the greatly enhanced renin inhibitory activity of angiotensinogen analogues bearing a reduced peptide bond is due to the change of stereochemistry at the scissile bond: the —CH<sub>2</sub>NH— moiety is isosteric with the tetrahedral transition state formed during amide hydrolysis and is therefore bound to the enzyme more tightly than the substrate<sup>16</sup>. As the reduced analogue is not cleaved by renin, it acts as a potent competitive inhibitor.

Because transition-state isosteres bind to the enzyme so much more tightly, the effect of structural variations present in substrates of different species is magnified, resulting in the large difference in binding to human compared with dog renin that we have observed.

**Table 3** Inhibition of cathepsin D, renal acid protease and renal renin by H-113 and H-142

| Inhibitor | Concentration (μM) | % Inhibition of Cathepsin D | % Inhibition of renal acid protease | Renal renin |
|-----------|--------------------|-----------------------------|-------------------------------------|-------------|
| H-113     | 6.0                | 0                           | 0                                   | 100         |
| H-142     | 712.0              | 0                           | 0                                   | 100         |
|           | 7.0                | 0                           | 0                                   | 100         |
|           | 0.007              | 0                           | 0                                   | 33          |

Cathepsin D and renal acid protease activities were measured by the hydrolysis of haemoglobin at pH 3.3 (ref. 17). Renal renin activity was measured using ox substrate<sup>28</sup>. The IC<sub>50</sub> of H-142 against renal renin was 0.01 ± 0.003 μM (n = 3), similar to that against plasma renin (0.01 ± 0.002 μM, n = 4).

Cathepsin D is another acid protease capable of producing angiotensin I from tetradecapeptide renin substrate 1 (ref. 17). We tested the ability of analogues H-113 and H-142 to inhibit pure human cathepsin D as well as 'acid protease' and renin from human kidney. We found that the reduced analogues are extremely poor inhibitors of both cathepsin D and renal 'acid protease'; thus they are highly specific for renin (Table 3).

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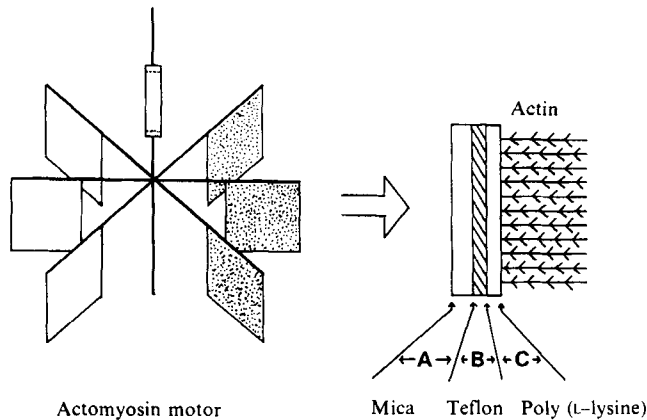
## An actomyosin motor

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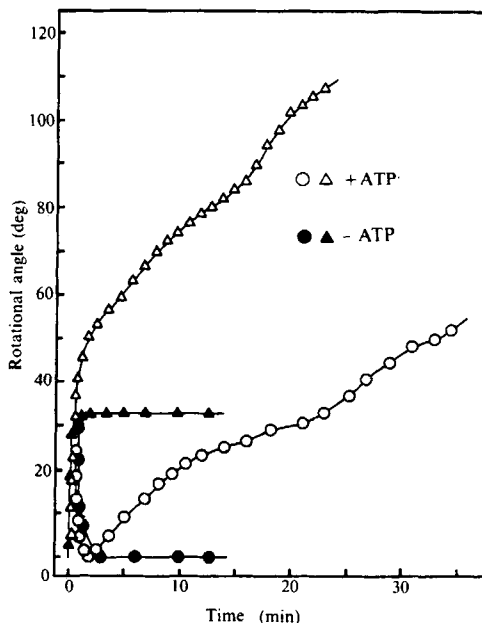
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Recently, two of us (M.Y. and H.S.) described an apparatus, termed the stream cell, in which a circulating flow could be induced by the interaction of heavy meromyosin (HMM) with F-actin (filamentous actin), purified from rabbit skeletal muscle<sup>1–5</sup>. Here we describe a novel system, the actomyosin motor, consisting of a rotor with F-actin attached which rotates in a specific direction, driven by the ATP-hydrolysing interaction of HMM with F-actin. This actomyosin motor can be prepared much more easily than the stream cell, and motor activity was observed with almost 100% success when the experiment was done carefully. This system represents a novel biological motor which, like the stream cell, is suitable for studying the chemo-mechanical coupling of actin and active myosin fragments.

The structure of the rotor is shown schematically in Fig. 1; it has an axis of rotation on which rests a float and three transverse bars to which blades (or vanes) are attached with wax. The axis and transverse bars are made of glass capillaries. The blades at each end of the transverse bars consist of a thin



**Fig. 1** A schematic representation of the rotor. The rotation axis and the three transverse bars are 4.0 cm long glass capillaries of  $\sim 0.3$  mm diameter, fixed to each other with wax. A blade of mica ( $1 \times 1$  cm,  $50 \mu\text{m}$  thick; A) is fixed to each end of the transverse bars. B denotes a thin sheet of Teflon  $\sim 10 \mu\text{m}$  thick. The surface of the Teflon sheet is further coated with poly(L-lysine) (C). Partial coating of the other side of the blade strongly interferes with rotation. The float consists of a polyethylene pipe of 2.7 and 3 mm inner and outer diameter, respectively, about 1.5 cm long, fixed to the rotation axis with wax. F-actin was fixed to the surface of the blade in the direction of the arrowheads. The blades were placed in a poly(L-lysine) solution ( $5 \text{ mg ml}^{-1}$ ,  $0.4 \text{ M KOH}$ ) at  $0^\circ\text{C}$  for 3 h and then washed well with a solution of  $0.1 \text{ M KCl}$ ,  $0.5 \text{ mM MgCl}_2$ ,  $2 \text{ mM imidazole buffer (pH 7.6)}$ . They were then immersed in a G-actin solution ( $0.25 \text{ mg ml}^{-1}$  G-actin,  $1 \text{ mM MgCl}_2$ ,  $2 \text{ mM imidazole buffer pH 7.6}$ ,  $0.2 \text{ mM ATP}$ ), and G-actin polymerization was initiated by adding a 20% (v/v) KCl solution ( $0.5 \text{ M KCl}$ ,  $1 \text{ mM MgCl}_2$ ,  $2 \text{ mM imidazole buffer}$ ). After incubation at  $20^\circ\text{C}$  for 1 h about  $6 \mu\text{g cm}^{-2}$  F-actin was fixed to the surface of the blades.

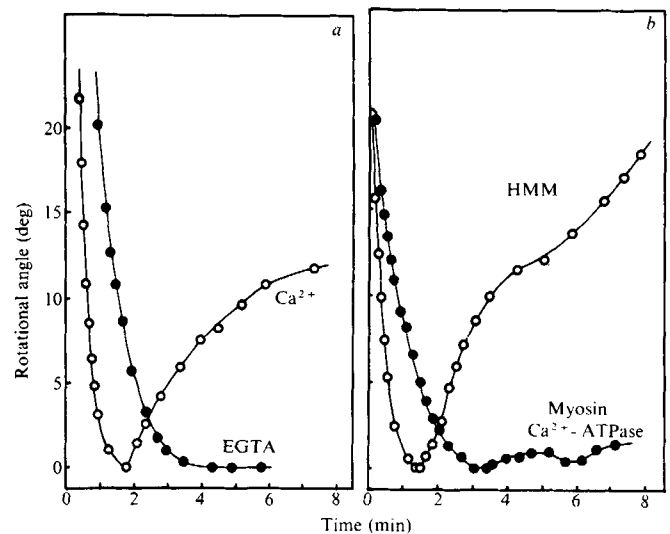


**Fig. 2** Rotations of the actomyosin motor in the presence (open symbols) and absence (solid symbols) of ATP when rotation was induced in the same (triangles) or opposite (circles) direction to the spontaneous direction. The ordinate denotes the angle between one of the blades and the axis of a space-fixed coordinate system, the origin of which lies on the axis of rotation. The solution contains  $2.0 \text{ mg ml}^{-1}$  HMM,  $90 \text{ mM KCl}$ ,  $4 \text{ mM MgCl}_2$ ,  $10 \text{ mM MOPS pH 7.0}$ , and  $30 \mu\text{M CaCl}_2$ . Open symbols represent results observed in the presence of  $2 \text{ mM ATP}$ .

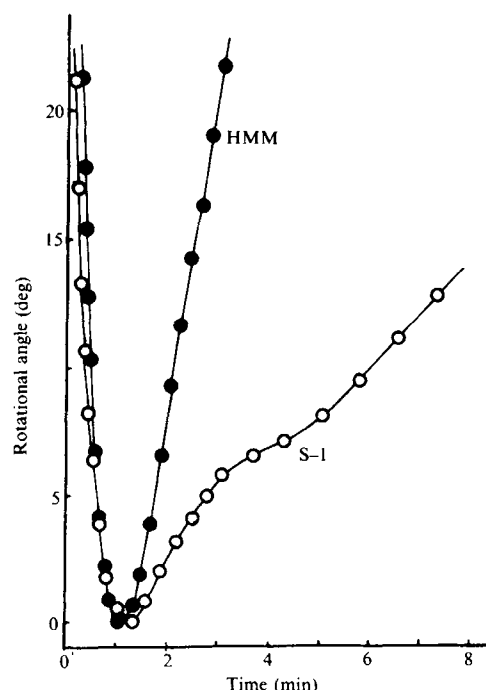
sheet of mica, with one side coated with Teflon. The layer of Teflon is further coated with poly(L-lysine). The size of the float is adjusted to suspend the rotor in Mg-ATP solution containing active myosin fragments. The rotor, total weight  $\sim 300 \text{ mg}$ , must be well balanced around the centre of rotation, otherwise it tends to drift during the observations.

Brown and Spudich<sup>6</sup> found that G-actin purified from *Dictyostelium discoideum* polymerizes on poly(L-lysine) with a specific polarity. We found that in suitable conditions, G-actin of rabbit skeletal muscle polymerizes in a similar manner on the surface of poly(L-lysine)-coated Teflon, which was verified in the electron microscope by observing the arrowhead structure of HMM and F-actin. This technique was applied to fix F-actin to the blades of the rotor with a specific polarity (as indicated by the arrowheads in Fig. 1). The movement of the rotor was observed from above with a stereomicroscope (Olympus type X;  $\times 80$ ) at  $20^\circ\text{C}$  and recorded with a video camera (Ikegami CTC-2100) connected to a video recorder.

In the presence of sufficient HMM and ATP, the rotor started spontaneously to rotate in the direction opposite to the side of the blades carrying attached F-actin, that is, in the direction indicated by the actin arrowheads in Fig. 1. Thus, HMM pushes



**Fig. 3** *a*, Calcium regulation of rotation. The ordinate and abscissa are the same as in Fig. 2. The solution contained  $2.0 \text{ mg ml}^{-1}$  HMM,  $90 \text{ mM KCl}$ ,  $2 \text{ mM ATP}$ ,  $4 \text{ mM MgCl}_2$ ,  $10 \text{ mM MOPS pH 7.0}$ .  $\text{Ca}^{2+}$  ( $30 \mu\text{M}$ ) or EGTA ( $1 \text{ mM}$ ) was added as required. *b*, The effect of heat produced by ATP hydrolysis on rotation of the motor (see text for detailed explanation). The solution was the same as that in *a* except that the HMM concentration was only  $0.1 \text{ mg ml}^{-1}$ . F-actin-activated ATPase activity on the surface of the blade was  $0.15 \text{ nmol P}_i$  per min per blade. Solid circles, myosin alone was fixed to the surface of the blades as follows: microcrystalline cellulose was uniformly fixed to activated cellulose powders to avoid the denaturation of myosin due to direct interactions with active sites. The activated blades were immersed in a LMM solution containing  $5 \text{ mg ml}^{-1}$  LMM,  $0.4 \text{ M KCl}$ ,  $10 \text{ mM Tris-HCl (pH 7.4)}$  at  $0^\circ\text{C}$  for 30 min. After washing several times with a buffer solution ( $50 \text{ mM KCl}$ ,  $10 \text{ mM MOPS pH 7.0}$ ), they were immersed in a solution of soluble myosin ( $5 \text{ mg ml}^{-1}$  myosin,  $0.4 \text{ M KCl}$ ,  $50 \text{ mM MOPS pH 7.0}$ ). The ionic concentration of the solution was gradually reduced by adding distilled water. At low ionic concentrations, myosin assumed a filamentous form which, incorporated with LMM, attached to the blades. The total protein (LMM+myosin) fixed per blade was  $\sim 20 \mu\text{g}$ . The  $\text{Ca}^{2+}$ -ATPase activity of the fixed myosin was measured in a solution containing  $50 \text{ mM KCl}$ ,  $1 \text{ mM CaCl}_2$ ,  $10 \text{ mM MOPS pH 7.0}$ ,  $2 \text{ mM ATP}$ . In the measurements of rotor movement,  $0.9\%$  sucrose was added to the solution to adjust the viscosity to the level used in the previous experiment. The  $\text{Ca}^{2+}$ -ATPase activity of the fixed myosin was  $1.8 \text{ nmol P}_i$  per min per blade.



**Fig. 4** Spontaneous rotation of the actomyosin motor produced by S-1. ●, Rotation produced by HMM, which is essentially the same as that indicated by open circles in Fig. 3b, except that the HMM concentration in this experiment was  $1.0 \text{ mg ml}^{-1}$ . ○, Rotation observed when S-1 replaced HMM. The solution consisted of  $2.5 \text{ mg ml}^{-1}$  S-1,  $2 \text{ mM}$  ATP,  $4 \text{ mM}$   $\text{MgCl}_2$ ,  $10 \text{ mM}$  MOPS pH 7.0,  $30 \text{ }\mu\text{M}$   $\text{CaCl}_2$ ,  $45 \text{ mM}$  KCl.

F-actin in the direction of the blade probably by moving the fluid in the opposite direction, the same direction of motion as that of cross-bridge movement on muscle fibre thin filaments. It was difficult to stop rotor movement completely before spontaneous rotation, so to try to create the same initial conditions for each experiment a slight rotation of the rotor was first given manually in the opposite direction to that of spontaneous rotation. This rotation stopped within several minutes (Fig. 2, open circles), and then spontaneous rotation began in the opposite direction and continued for 30–40 min. This spontaneous rotation was not observed in the absence of ATP (closed circles in Fig. 2). Note that the spontaneous rotations occurred in one specific direction, irrespective of the direction of the initial rotation. The spontaneous rotation was regulated by calcium if troponin and tropomyosin were attached to actin filaments on the blade. Figure 3 shows the rotation in the presence and absence of calcium (open and closed circles, respectively).

The system must be carefully arranged to prevent convection, which may move the blades. We found that continuous illumination from the top of the system with a tungsten light was effective in suppressing convection caused by evaporation from the surface of the fluid. In our motor the side of the blades responsible for the spontaneous rotation tends to be hotter than the other side due to heat liberated by the actomyosin-ATPase activity. Brownian movements of molecules will be more pronounced on the hotter than the colder side, therefore the former might push the latter, giving rise to rotation of the rotor in the spontaneous direction. The following results show that this is not the case for the actomyosin system. Figure 3b (open circles) shows the spontaneous rotation of the rotor, which is essentially the same as that described above; closed circles denote the rotation when myosin alone was fixed to the specific side of the blades instead of F-actin. In the latter case, the  $\text{Ca}^{2+}$ -ATPase activity of the fixed myosin was adjusted to be about 12 times larger than the acto-HMM-ATPase activity in the previous experiment. Therefore, the temperature difference between the two sides of the blades should be considerably larger than in the case of acto-HMM-ATPase. As shown in Fig. 3, only small,

irregular movements of the rotor were observed after the decay of the initial rotation for myosin alone. However, such movements were clearly distinguishable from the large and definite spontaneous rotation (denoted by open circles).

Thus we conclude that the spontaneous rotation of the actomyosin motor is active and driven by ATPase. The present results are consistent with those obtained for our stream cell where HMM induces streaming in the direction of the tail of the arrowhead. In a further experiment, we replaced HMM in the Mg-ATP solution with subfragment-1 (S-1), prepared from myosin by chymotryptic digestion. The purity of the preparation was checked by SDS-polyacrylamide gel electrophoresis. We found that S-1, together with F-actin, can also induce rotation of the motor (Fig. 4).

Details of the preparation of the actomyosin motor will be reported elsewhere.

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## Unusual association of V, J and C regions in a mouse immunoglobulin $\lambda$ chain

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The light chains of mouse immunoglobulins have been classified into two main groups,  $\kappa$  and  $\lambda$ . The majority (>90%) are  $\kappa^{1-3}$  and share a single constant (C) region; however, they differ extensively in their variable (V) regions (for example, ref. 4). The lambda group consists of three subtypes,  $\lambda 1$ ,  $\lambda 2$  and  $\lambda 3$  (refs 5–7), which are classified according to the amino acid sequence of the C region. Each C region (for example,  $C_{\lambda 1}$ ) is encoded by a distinct gene<sup>8–11</sup>, which carries on its 5' side a J gene segment<sup>9–13</sup> (for example,  $J_{\lambda 1}$ ). A fourth gene pair ( $J_{\lambda 4}C_{\lambda 4}$ ) is probably not expressed<sup>12,13</sup>.  $J_{\lambda 3}C_{\lambda 3}$  is closely linked to  $J_{\lambda 1}C_{\lambda 1}$  in one cluster<sup>10,11</sup> and  $J_{\lambda 2}C_{\lambda 2}$  to  $J_{\lambda 4}C_{\lambda 4}$  in a second cluster<sup>10</sup>, on chromosome 16 (ref. 14). Only two genes ( $V_{\lambda 1}$  and  $V_{\lambda 2}$ ) encode the  $\lambda$  V regions<sup>8,9,15</sup>. To date,  $V_{\lambda 1}$  has been found only in association with  $J_{\lambda 1}C_{\lambda 1}$  (in  $\lambda 1$  chains)<sup>5,16</sup> or  $J_{\lambda 3}C_{\lambda 3}$  (in  $\lambda 3$  chains)<sup>19</sup> and  $V_{\lambda 2}$  only with  $J_{\lambda 2}C_{\lambda 2}$  (in  $\lambda 2$  chains)<sup>6,17,18</sup>. It has been argued that  $V_{\lambda 1}$  is probably located 5' to the  $J_{\lambda 3}C_{\lambda 3}J_{\lambda 1}C_{\lambda 1}$  cluster and  $V_{\lambda 2}$  5' to the  $J_{\lambda 2}C_{\lambda 2}J_{\lambda 4}C_{\lambda 4}$  cluster<sup>10</sup>. The exclusive recombination of each  $V_{\lambda}$  with the nearest  $J_{\lambda}C_{\lambda}$  cluster on its 3' side would provide a genetic basis for restriction in V–C association. However, such restriction does not appear to be an invariant feature of  $\lambda$  chain structure, for here we describe an unusual chain in which  $V_{\lambda 2}$  is associated with  $J_{\lambda 3}C_{\lambda 3}$ .

The light chain of the monoclonal anti-2,4-dinitrophenyl (DNP) antibody 6-2 ( $L^{6-2}$ ) was provisionally typed serologically as  $\lambda 2$  (R. M. Breyer and H. N. E., unpublished observations). Its amino acid composition, however, was found to differ significantly from that specified by the germ-line  $V_{\lambda 2}$ ,  $J_{\lambda 2}$  and  $C_{\lambda 2}$  genes (Table 1). This difference was unexpected as the  $\lambda$  chains previously examined (three  $\lambda 2$  (refs 6, 17, 18), about 20  $\lambda 1$  (refs 5, 16), and a single  $\lambda 3$  (ref. 19)) had been found to be very similar in amino acid sequence to the respective prototype sequences (that is, those sequences encoded by germ-line genes). As controls, the amino acid compositions of light chains from myeloma protein HOPC-1 ( $\lambda 1$ ), and monoclonal antibodies 8-13 ( $\lambda 2$ ) and 5-8 ( $\lambda 3$ ) were determined; each agreed well with the composition of its prototype (Table 1). It therefore



seemed possible that  $L^{6-2}$  was encoded by an unusual combination of genes. Indeed, its amino acid composition approximated that expected from a chain encoded by germ-line  $V_{\lambda 2}$ ,  $J_{\lambda 3}$  and  $C_{\lambda 3}$  (Table 1). It agreed less well with the compositions corresponding to other combinations of germ-line genes (for example,  $V_{\lambda 2}J_{\lambda 1}C_{\lambda 1}$ ).

To obtain further evidence of the structure of  $L^{6-2}$ , we have carried out a partial amino acid sequence analysis. The isolated chain was extensively reduced, carboxymethylated, and reacted with citraconic anhydride to increase its solubility. It was then treated with pyroglutamyl aminopeptidase to remove the cyclized glutamyl residue that occurs at the  $NH_2$ -terminus of mouse  $\lambda$  chains<sup>5,6,16,18,19</sup>. Automated Edman degradation of the resulting protein established the sequence of residues 2–22. In this region, the prototype amino acid sequence of  $V_{\lambda 2}$  differs from that of  $V_{\lambda 1}$  at two positions, 16 and 19. At both of these positions, the residues found in  $L^{6-2}$  were identical to those in the  $V_{\lambda 2}$  prototype (glycine and isoleucine) and differed from those in the  $V_{\lambda 1}$  prototype (glutamic acid and threonine) (Fig. 1).

Additional sequence information was obtained by digesting reduced, carboxymethylated  $L^{6-2}$  with cyanogen bromide and subjecting the unfractionated digest to automated Edman degradation. As  $L^{6-2}$  has three methionyl residues and a blocked  $NH_2$ -terminus, phenylthiohydantoin(PTH)-amino acids derived from three fragments were expected at each cycle. Comparison of the mixed sequence with the various prototype

sequences of  $V_{\lambda 1}$ ,  $J_{\lambda}$  and  $C_{\lambda}$  identified which genes are likely to encode the  $L^{6-2}$  chain.

At the first cycle of the Edman degradation, PTH-Tyr, PTH-Phe and PTH-Ala were found. As indicated in Fig. 1, these are the residues expected if cleavage had occurred after methionyl residues 87 (in  $V_{\lambda 2}$ ), 120 (in  $C_{\lambda 3}$ ) and 175 (in  $C_{\lambda 1}$ ,  $C_{\lambda 2}$  or  $C_{\lambda 3}$ ). The mixture of PTH-amino acids found at subsequent cycles was consistent with these cleavage sites. Since only  $\lambda 3$  chains have a methionyl residue at position 120, these results indicate that the C region of  $L^{6-2}$  is  $C_{\lambda 3}$ . This assignment was confirmed by the presence of PTH-Pro, not PTH-Ser, at cycle 5 of the mixed sequence (position 125). At cycle 9, PTH-Gln was found, as in  $C_{\lambda 3}$  (position 129); although PTH-Thr was also present at this cycle, it was presumably derived from a threonyl residue at position 96 (see below). The remaining positions in the mixed sequence were consistent with the  $C_{\lambda 3}$  sequence, but only the three positions mentioned (120, 125 and 129) distinguished  $C_{\lambda 3}$  from  $C_{\lambda 1}$  and  $C_{\lambda 2}$ . No information was obtained concerning residues in  $L^{6-2}$  at the two other positions (171 and 174) where  $C_{\lambda 2}$  and  $C_{\lambda 3}$  differ<sup>7</sup>.

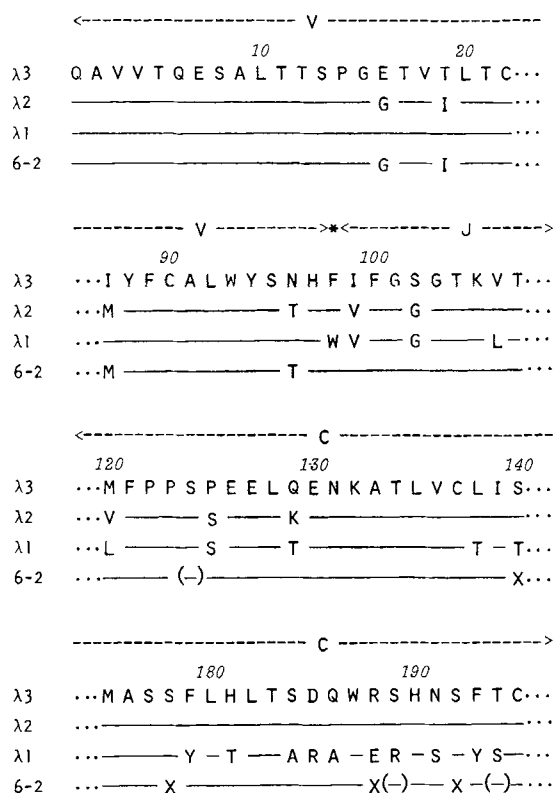
It is more difficult to classify the V and J regions of  $L^{6-2}$  as the corresponding genes are subject to somatic mutation. Thus, a change in a single base pair could have converted the isoleucyl residue at position 87 in the  $V_{\lambda 1}$  prototype into a methionyl residue in  $L^{6-2}$ . However, at cycle 9, the mixed sequence showed PTH-Thr, not PTH-Asn, again in agreement with the prototype sequence of  $V_{\lambda 2}$  and not that of  $V_{\lambda 1}$  at position 96. Moreover,

Table 1 Amino acid compositions of mouse  $\lambda$  chains

|        | 6-2      |                                                       | HOPC-1   |                                                       | 8-13     |                                                       | 5-8      |                                                       |
|--------|----------|-------------------------------------------------------|----------|-------------------------------------------------------|----------|-------------------------------------------------------|----------|-------------------------------------------------------|
|        | Observed | Expected<br>$V_{\lambda 2}J_{\lambda 3}C_{\lambda 3}$ | Observed | Expected<br>$V_{\lambda 1}J_{\lambda 1}C_{\lambda 1}$ | Observed | Expected<br>$V_{\lambda 2}J_{\lambda 2}C_{\lambda 2}$ | Observed | Expected<br>$V_{\lambda 1}J_{\lambda 3}C_{\lambda 3}$ |
| CmCys* | 5.0      | 5                                                     | 4.3      | 5                                                     | 4.7      | 5                                                     | 4.9      | 5                                                     |
| Asp    | 18.2     | 17                                                    | 15.2     | 15                                                    | 16.3     | 16                                                    | 18.4     | 18                                                    |
| Thr    | 27.9     | 29                                                    | 29.5     | 30                                                    | 28.7     | 29                                                    | 27.7     | 29                                                    |
| Ser    | 22.9     | 23                                                    | 22.9     | 23                                                    | 23.1     | 23                                                    | 22.2     | 22                                                    |
| Glu    | 19.8     | 19                                                    | 21.7     | 21                                                    | 18.5     | 18                                                    | 21.7     | 21                                                    |
| Pro    | 13.4     | 13                                                    | 11.3     | 11                                                    | 12.0     | 12                                                    | 13.2     | 13                                                    |
| Gly    | 16.6     | 17                                                    | 17.6     | 17                                                    | 19.0     | 19                                                    | 16.0     | 16                                                    |
| Ala    | 14.8     | 15                                                    | 15.9     | 16                                                    | 14.9     | 15                                                    | 15.8     | 16                                                    |
| Val    | 15.0     | 15                                                    | 16.9     | 17                                                    | 16.6     | 17                                                    | 14.1     | 14                                                    |
| Met    | 3.0      | 3                                                     | 2.0      | 2                                                     | 2.1      | 2                                                     | 2.1      | 2                                                     |
| Ile    | 6.9      | 7                                                     | 4.9      | 5                                                     | 5.8      | 6                                                     | 6.9      | 7                                                     |
| Leu    | 15.6     | 16                                                    | 15.0     | 15                                                    | 15.8     | 16                                                    | 15.8     | 16                                                    |
| Tyr    | 4.2      | 4                                                     | 6.6      | 7                                                     | 3.1      | 3                                                     | 4.2      | 4                                                     |
| Phe    | 8.6      | 9                                                     | 5.8      | 6                                                     | 9.9      | 10                                                    | 8.7      | 9                                                     |
| His    | 4.9      | 5                                                     | 5.0      | 5                                                     | 4.9      | 5                                                     | 4.9      | 5                                                     |
| Lys    | 8.7      | 9                                                     | 8.8      | 9                                                     | 9.9      | 10                                                    | 8.8      | 9                                                     |
| Arg    | 4.6      | 4                                                     | 5.9      | 6                                                     | 4.2      | 4                                                     | 4.5      | 4                                                     |
| Trp    | ND       | 4                                                     | ND       | 5                                                     | ND       | 4                                                     | ND       | 4                                                     |
| Total  |          | 214                                                   |          | 215                                                   |          | 214                                                   |          | 214                                                   |

Monoclonal antibodies 6-2 ( $\mu$ ,  $\lambda$ ) and 5-8 ( $\mu$ ,  $\lambda 3$ ) were from a panel of  $\lambda 2$ - or  $\lambda 3$ -producing hybridomas prepared by fusing the myeloma Sp2/0-Ag 14 with spleen cells of mice immunized with DNP-N-(2-aminoethyl)carbamylmethylated Ficoll (R. Zaugg and H.N.E., unpublished experiments). Monoclonal antibody 8-13 ( $\gamma 1$ ,  $\lambda 2$ ) was a gift from A. Marshak-Rothstein and M. L. Gefter and has been described elsewhere<sup>7</sup>. The antibodies were isolated by affinity chromatography with DNP-lysyl Sepharose 4B. After partial reduction and alkylation with iodoacetic acid or iodoacetamide<sup>23</sup>, the light and heavy chains were separated by gel filtration with Sephadex G-100 in 6 M urea, 1 M acetic acid. The light chain of myeloma HOPC-1 ( $\gamma 2a$ ,  $\lambda 1$ ), a gift from T. Imanishi-Kari, was prepared in a similar manner. After extensive reduction and alkylation<sup>23</sup>, duplicate or triplicate samples of light chains (0.9 nmol) were hydrolysed in constant-boiling HCl for 24, 48 and 72 h, and applied to a Dionex D-500 amino acid analyser. The analyser was calibrated with 10 nmol of a mixture of standard amino acids; to determine the linearity, standards were also applied in amounts ranging from 2.5 to 20 nmol. The experimental values were corrected for deviations from linearity that exceeded 2% (the maximum deviation was 6%) and were normalised to the expected number of residues in each chain, not including threonine, serine, valine, isoleucine, methionine, half-cystine and tryptophan. The values shown are averages of samples at each time point except for valine and isoleucine, which are the 72 h values, and serine and threonine, which were determined by logarithmic extrapolation to time 0. The resulting values were renormalized to 205 residues (the expected number of residues in each chain less half-cystine and tryptophan). The light chains derived from IgM antibodies 6-2 and 5-8 contained a small amount of J chain ( $\sim 0.03$  mol J per mol L), as determined by alkaline urea polyacrylamide gel electrophoresis. Compared to light chain, J chain is rich in arginine<sup>24,25</sup>. We calculated that the presence of the J chain would contribute 0.1–0.2 residue to the determined content of arginine. Expected values were calculated from prototype amino acid sequences. The sequences used for positions 1–97 correspond to the germ-line DNA sequences of  $V_{\lambda 1}$  (ref. 9) and  $V_{\lambda 2}$  (ref. 15), those for positions 99–110 correspond to the germ-line DNA sequences of  $J_{\lambda 1}$ ,  $J_{\lambda 2}$  and  $J_{\lambda 3}$  (refs 12, 13), and those for positions 111–214 or 215 correspond to the germ-line DNA sequences of  $C_{\lambda 1}$  (ref. 26) and  $C_{\lambda 2}$  (ref. 27) and to the actual amino acid sequence of  $C_{\lambda 3}$  (refs 7, 19). Position 98 is encoded by the 3' end of  $V_{\lambda}$  and/or the 5' end of  $J_{\lambda}$  (ref. 12). We assume here that position 98 in  $\lambda 1$  chains is tryptophan (encoded by  $V_{\lambda 1}/J_{\lambda 1}$  or  $J_{\lambda 1}$ ), in  $\lambda 2$  chains phenylalanine (encoded by  $V_{\lambda 2}$  or  $V_{\lambda 2}/J_{\lambda 2}$ ), and in  $\lambda 3$  chains phenylalanine (encoded by  $V_{\lambda 1}/J_{\lambda 3}$  or  $J_{\lambda 3}$ ). These assignments agree with the residues found most frequently at position 98 of  $\lambda 1$ ,  $\lambda 2$  and  $\lambda 3$  chains. In calculating the expected composition of  $L^{6-2}$ ,  $V_{\lambda 2}$  was assumed to encode positions 1–97 and  $J_{\lambda 3}$  positions 99–110; position 98 is phenylalanine whether encoded by  $V_{\lambda 2}$  and/or  $J_{\lambda 3}$ . The amino acid sequence of the classical V region of the light chain of HOPC-1 (ref. 28) corresponds to the  $V_{\lambda 1}-J_{\lambda 1}$  gene segments with tryptophan at position 98. The amino acid sequence predicted from the DNA sequence of  $C_{\lambda 1}$  (ref. 26) differs from the amino acid sequence reported for  $C_{\lambda 1}$  from protein MOPC 104E (ref. 5) at several positions and, in total, contains one more residue of alanine and one less of serine. The alanine and serine content determined here for the light chain of HOPC-1 agrees with the DNA sequence of  $C_{\lambda 1}$  (together with that of germ-line  $V_{\lambda 1}$  and  $J_{\lambda 1}$ ). The amino acid sequence of the V region of  $L^{8-13}$  (refs 17, 18 and our unpublished observations) corresponds to the  $V_{\lambda 2}-J_{\lambda 2}$  gene segments with phenylalanine at position 98. The amino acid sequence of 100/110 residues of the V region of  $L^{5-8}$  has been determined<sup>19</sup> and corresponds to the DNA sequence of  $V_{\lambda 1}-J_{\lambda 3}$ . ND, not determined.

\* S-carboxymethylcysteine.



**Fig. 1** Partial amino acid sequence of L<sup>6-2</sup> compared with prototype sequences of λ<sub>1</sub>, λ<sub>2</sub> and λ<sub>3</sub> chains (see Table 1 legend for description of prototype sequences, with references). Position 98(\*) is encoded by V<sub>λ</sub> and/or J<sub>λ</sub> (ref. 12). Solid lines indicate identity in amino acid sequence with the λ<sub>3</sub> prototype sequence shown in the top line. Positions 1-22: L<sup>6-2</sup> was extensively reduced and alkylated with [1-<sup>14</sup>C]iodoacetic acid. An aliquot (20 nmol) was citraconylated<sup>29</sup> and treated with pyroglutaminyl aminopeptidase<sup>30</sup>. The residue at position 1, which is removed by this enzyme, is presumably cyclized glutamine and is designated Q to conform to the prototype sequences. The unblocked sample was subjected to automated Edman degradation in a Beckman 890C sequencer with a 0.1 M Quadrol program<sup>31</sup>. Positions 88-107, 121-140 and 176-195: another aliquot of extensively reduced, alkylated L<sup>6-2</sup> (50 nmol) was treated with 53 mg CNBr in 1.3 ml 70% formic acid for 24 h at room temperature; after freeze-drying, 25 nmol of the digest was subjected to automated Edman degradation in the presence of 6 mg Polybrene<sup>32</sup>. The PTH derivatives were identified (up to three per cycle) and the amino acids were assigned to the λ<sub>1</sub>, λ<sub>2</sub> or λ<sub>3</sub> sequences by homology with the prototype sequences. PTH-amino acids in the ethyl acetate phase were identified by GLC, HPLC, TLC and amino acid analysis (after back-hydrolysis in HI) as described elsewhere<sup>33</sup>. <sup>14</sup>C-labelled S-carboxymethylcysteine was identified by counting the ethyl acetate phase. Histidine was identified by amino acid analysis of the aqueous phase after back-hydrolysis. Arginine, which is expected at position 188, was not identified by amino acid analysis because a contaminant in the aqueous phase, presumably derived from one of the sequencer chemicals, co-eluted with this amino acid. Serine could not be identified when S-carboxymethylcysteine was present at the same cycle because the two PTH derivatives have the same retention time on GLC and both are converted to alanine on back-hydrolysis; the identification of serine by HPLC was not reliable. Residues in parentheses denote tentative identifications; X indicates no identification. Positions 87, 120 and 175 were assumed to be methionine, based on the results of CNBr cleavage.

as discussed previously, at two other discriminating positions (16 and 19) the L<sup>6-2</sup> sequence agreed with the prototype sequence of V<sub>λ2</sub>. Coincidental changes at four positions (16, 19, 87 and 96) from the V<sub>λ1</sub> to the V<sub>λ2</sub> prototype sequence are unlikely to result from somatic mutation. We have no information about the residues in L<sup>6-2</sup> at the three other positions (54, 62 and 85) where the two V<sub>λ</sub> prototype sequences differ.

Positions 99 to 110 of λ chains are encoded by the J<sub>λ</sub> gene segments; position 98 is encoded by nucleotides from the 3' end of V<sub>λ</sub>, the 5' end of J<sub>λ</sub>, or by a combination of these<sup>12</sup>. The presence of PTH-Val at cycle 19 (position 106) is consistent with the prototype sequence of J<sub>λ2</sub> and J<sub>λ3</sub>, but not that of J<sub>λ1</sub> (refs 12, 13). At cycle 12 (position 99), the presence of PTH-Ile and the absence of PTH-Val agrees with J<sub>λ3</sub>, not J<sub>λ2</sub> or J<sub>λ1</sub>. Similarly, at cycle 15 (position 102), the presence of PTH-Ser

instead of PTH-Gly agrees only with J<sub>λ3</sub>. There are no other positions in the J segments that discriminate between the J<sub>λ3</sub> and J<sub>λ2</sub> prototype sequences.

We have presented evidence, based on amino acid sequence and composition data, that L<sup>6-2</sup> is encoded by V<sub>λ2</sub>, J<sub>λ3</sub> and C<sub>λ3</sub>. This conclusion is supported by the finding (E. B. Reilly and B. Blomberg, unpublished experiments) that DNA from the 6-2 hybridoma yields a distinctive EcoRI restriction fragment consistent with a V<sub>λ2</sub>-J<sub>λ3</sub> rearrangement, but not with any other V<sub>λ</sub>-J<sub>λ</sub> combination<sup>10</sup>. Analyses of DNA from a panel of hybridomas expressing various λ chains have so far not revealed another example of this rearrangement (E. B. Reilly, B. Blomberg, T. Imanishi-Kari, S. Tonegawa and H.N.E., unpublished observations).

The association of V<sub>λ1</sub> and V<sub>λ2</sub> with the J<sub>λ</sub>C<sub>λ</sub> genes seems to be nonrandom. For example, V<sub>λ1</sub> is expressed more frequently in association with J<sub>λ1</sub>C<sub>λ1</sub> than with J<sub>λ3</sub>C<sub>λ3</sub> (ref. 20). Although this difference may reflect antigen selection or regulatory effects of T cells, it might also be a result of differential efficiency of V-J joining<sup>12,13</sup>. However, the regulation of the joining of one V<sub>λ</sub> (for example, V<sub>λ1</sub>) with a J gene segment within a single cluster (for example, J<sub>λ3</sub> or J<sub>λ1</sub>) may differ from the regulation of the joining of that same V<sub>λ</sub> with a J gene segment in the other cluster (for example, J<sub>λ2</sub>). The location of V<sub>λ1</sub> and V<sub>λ2</sub> within chromosome 16 is unknown. Blomberg *et al.*<sup>10</sup> have obtained evidence suggesting that V<sub>λ1</sub> is 5' to the J<sub>λ3</sub>C<sub>λ3</sub>J<sub>λ1</sub>C<sub>λ1</sub> cluster and V<sub>λ2</sub> is 5' to the J<sub>λ2</sub>C<sub>λ2</sub>J<sub>λ4</sub>C<sub>λ4</sub> cluster. Their argument is based on the assumption that V<sub>λ</sub>-J<sub>λ</sub> joining requires deletion of intervening DNA<sup>21,22</sup>. Such an arrangement might facilitate the joining of each V<sub>λ</sub> with a J<sub>λ</sub> in the nearest downstream cluster; infrequently, a V<sub>λ</sub> (for example, V<sub>λ2</sub>) may combine with one of the J<sub>λ</sub> gene segments in the distant cluster (for example, J<sub>λ3</sub>). In an extension of this argument, the data presented here are consistent with a gene arrangement in which the (V<sub>λ2</sub>)J<sub>λ2</sub>C<sub>λ2</sub>J<sub>λ4</sub>C<sub>λ4</sub> cluster is 5' to the (V<sub>λ1</sub>)J<sub>λ3</sub>C<sub>λ3</sub>J<sub>λ1</sub>C<sub>λ1</sub> cluster. In this case, λ chains with V<sub>λ2</sub>J<sub>λ3</sub>C<sub>λ3</sub> and V<sub>λ2</sub>J<sub>λ1</sub>C<sub>λ1</sub>, but not with V<sub>λ1</sub>J<sub>λ2</sub>C<sub>λ2</sub> combinations, may occasionally be found.

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## A virus mutant with an insertion of a *copia*-like transposable element

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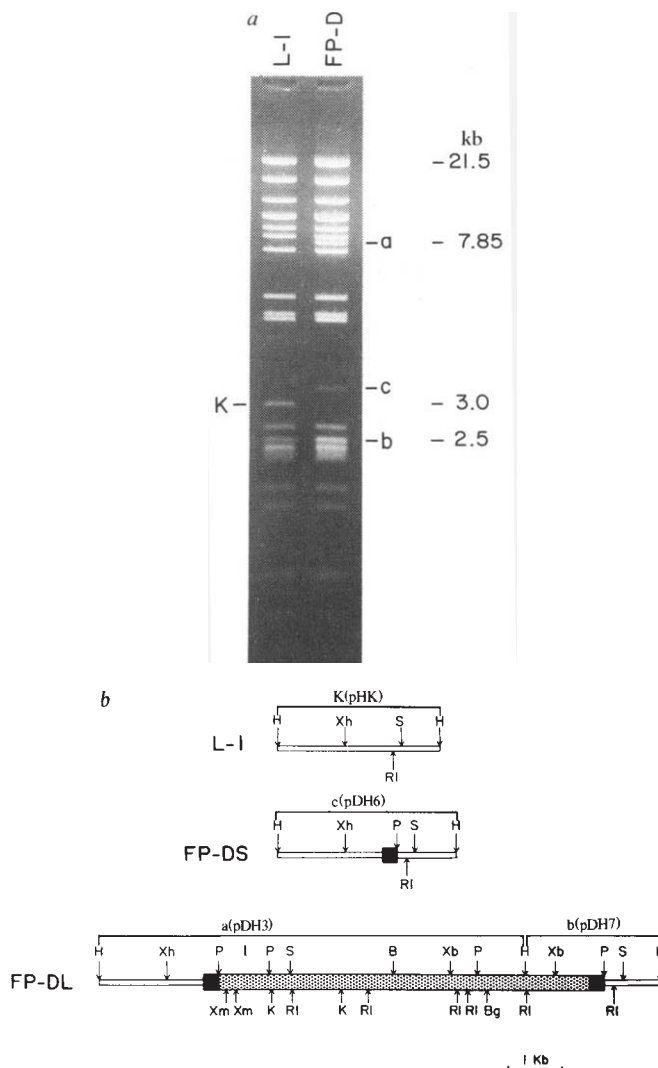
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The important role insect viruses have in the biological control of pests has inspired a considerable effort towards understanding their molecular biology. The nuclear polyhedrosis viruses (NPVs) are particularly interesting and accessible owing to their unique nuclear replication cycle and large (~100 kilobase, kb) double-stranded, circular DNA genome. Serial passage of NPVs in cell culture results in a gradual reduction in the ability of the virus to carry out the biphasic growth cycle of producing extracellular virus particles (non-occluded virus, NOV), followed by the occlusion of virus particles in proteinaceous polyhedra within the nucleus. Instead, NOV become the predominant replication product of serially passaged NPVs, with occlusion being a rarely observed, and probably aberrant, phenomenon<sup>1-5</sup>. Plaque purification of *Autographa californica* (the alfalfa looper, Lepidoptera; Noctuidae) NPV (AcNPV) after 25 passages in cell cultures of *Trichoplusia ni* (the cabbage looper, Lepidoptera; Noctuidae) resulted in the isolation of few-polyhedra (FP) mutants. One of these plaque isolates, FP-D, exhibited a restriction endonuclease fragment pattern indicative of an insertion, probably of non-viral DNA, at 86.4–86.6 on the physical map. Of additional interest was the observation of restriction fragments in sub-molar quantities<sup>6</sup>. We have now characterized this mutant; it contains an insertion of a *copia*-like transposable element, designated TED, derived from the host *T. ni* cells.

Figure 1a shows the *Hind*III pattern of AcNPV and FP-D DNAs. In the *Hind*III digest of FP-D, three new bands (a, b and c) are observed. Band c is clearly sub-molar. It is not a simple contaminant as it can survive several plaque purifications. The three bands appear with the concomitant loss of only one AcNPV fragment, *Hind*III-K.

A screen of a larger number of FP-D plaques yields two types of virus populations. The predominant type has an identical restriction endonuclease pattern to FP-D (*Hind*III a, b and sub-molar c). The other contains the *Hind*III c band (now molar) and has lost the a, b and K bands. This virus is designated FP-DS. We thus infer that FP-D is a mixture of two viruses: FP-DS and FP-DL, both of which contain an insert, but of differing size, in the *Hind*III-K region. FP-DL could not be plaque-purified free of FP-DS.

The nature of this genomic alteration was further clarified by cloning the *Hind*III bands a, b, c and K into *Escherichia coli* using pBR322 as a vector. Physical mapping of these cloned DNAs as well as the intact viral mutant DNA revealed that bands a and b are contiguous and together constitute the *Hind*III-K fragment; the *Hind*III-K fragment of FP-DL has a 7.3 kb insertion bearing an additional *Hind*III site. The *Hind*III c band is the *Hind*III-K fragment containing a 0.27 kb insertion. The physical maps of the *Hind*III-K fragment and its two different insertions (designated FP-DL and FP-DS) are shown in Fig. 1b. We have examined the origin of the 7.3 and 0.27 kb insertions by using the clones to probe genomic blots of both the virus and the host cell, *T. ni*. Only the viral *Hind*III-K fragment is probed with these clones (data not shown), indicating the sequences are non-viral in origin, as suggested by the physical mapping data. However, probing of the genome of uninfected cultured *T. ni* cells reveals a complex pattern of homology and demonstrates the origin as the *T. ni* host cells (Fig. 2). The intense bands present in the autoradiogram correspond to sequences found inside the FP-DL insert (see Fig. 1b). Fainter bands are also observed. We have cloned low-copy

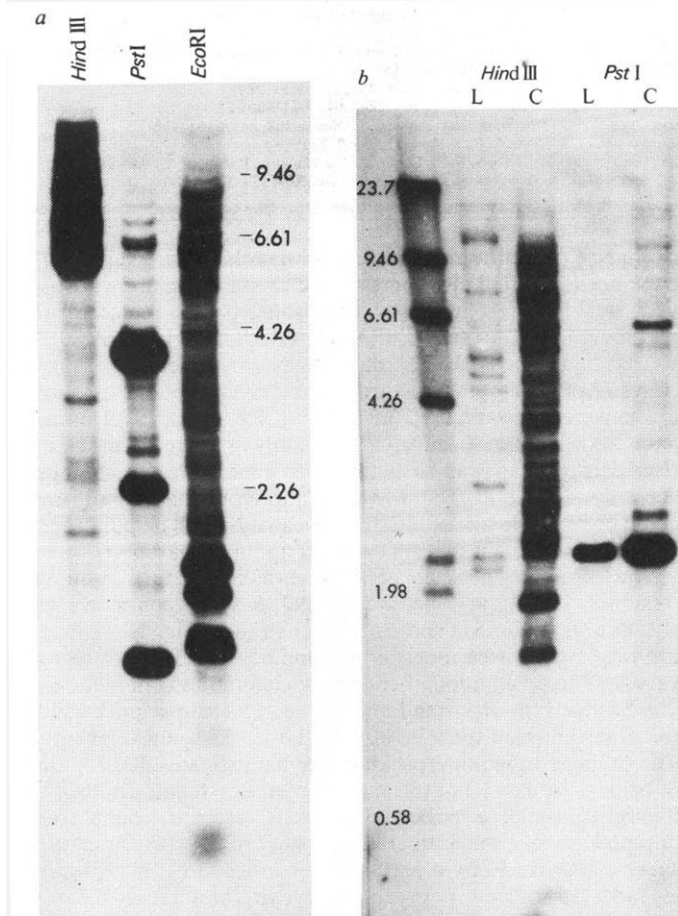


**Fig. 1** Comparison of AcNPV and FP-D DNAs. *a*, Ethidium bromide-stained 0.6% agarose gel, displaying *Hind*III digests of DNA from AcNPV, variant L-1<sup>22,23</sup> and NOV DNA from the mutant FP-D. NOV DNA was routinely prepared as follows: media (20 ml) from two 100 mm plates of infected *Spodoptera frugiperda* (IPLB-SF-21) cells were cleared at 1,700g for 15 min. The supernatant was further centrifuged for 90 min at 25,000 r.p.m. in the Beckman SW 27 rotor. The pellet was covered with 500  $\mu$ l of 5 mM NaCl, 10 mM EDTA, 0.1% SDS, 10 mM Tris-HCl, pH 7.8, and proteinase K was added to 100  $\mu$ g ml<sup>-1</sup>. Incubation at 37 °C for several hours resulted in complete dissolution of the pellet. The solution was extracted three times with phenol/chloroform/isoamyl alcohol/8-hydroxyquinoline (100 ml:100 ml:0.2 ml:0.2 g) and ethanol-precipitated. The AcNPV *Hind*III-K fragment and the new bands observed in the mutant FP-D, a, b and c, are indicated. Band b migrates at the same position as the AcNPV *Hind*III-M fragment. *b*, Physical map of the *Hind*III-K fragment and the different insertions present in the mutant FP-D (see text). The *Hind*III bands K, a, b and c were cloned into the *Hind*III site of pBR322 and mapped by standard techniques. Only the viral sequences of each plasmid are shown, and these are composed as they exist in their respective virus. The complete *Hind*III-K fragment is contained in clone pHK and is indicated L-1. Band c is contained entirely in pDH6 and indicated FP-DS. Band a is contained in pDH3 and extends from the left-most to the middle *Hind*III sites on FP-DL. Band b is contained in clone pDH7 and extends from the middle to the right-most *Hind*III sites on FP-DL. The stippled regions indicate the insertions present in FP-DL and FP-DS. An entire copy of the element TED is present in FP-DL. Heavy stippling demarcates the terminal repetitions, ID sequences, drawn relative to the *Pst*I sites as suggested by the hybridization data (see text and Fig. 3). H, *Hind*III; Xh, *Xho*I; RI, *Eco*RI; P, *Pst*I; S, *Sal*I; Xm, *Xma*I; K, *Kpn*I; B, *Bam*HI; Bg, *Bgl*II; Xb, *Xba*I.

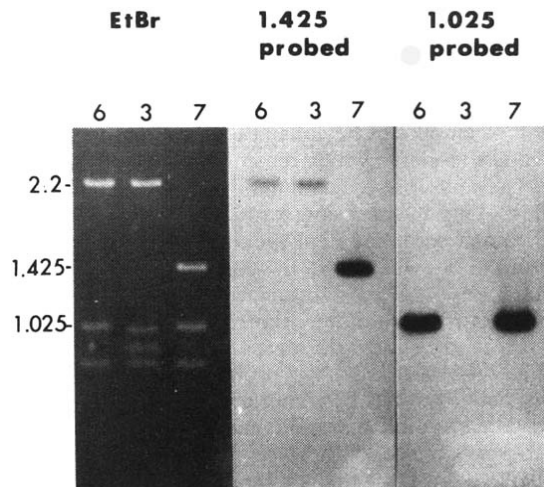


number sequences from the *T. ni* genome and find that, in quantitatively controlled experiments, they probe blots with the intensity of these fainter bands (data not shown). By analogy, then, with other systems, the insert is a middle repetitive sequence which is dispersed throughout the *T. ni* genome, the intense bands representing the largely conserved internal sequences and the fainter bands representing *T. ni* flanking sequences.

This sequence must be mobile since it has inserted into the viral genome; routine viral integration into the host chromosome is not observed in AcNPV infections (ref. 7 and our unpublished observations). The mobility of the sequence is also indicated by the difference in copy number and distribution of this sequence when DNA of the *T. ni* cell line is compared with DNA of *T. ni* larvae (Fig. 2b). As is evident from the *Hind*III-digested lanes, the sequence has greatly amplified in the cell line. When *Pst*I-digested larval genomic DNA is probed with pDH7 (see Fig. 1 legend), virtually all the label appears in a 2.3 kb fragment, as expected from the map of FP-DL. This, however, is not true for *Pst*I-digested cell line DNA where some label appears in fragments at 2.5 and 6.5 kb, indicating



**Fig. 2** Presence of TED in the *T. ni* genome. *a*, DNA from uninfected cultured *T. ni* cells (TN-368) was digested with the restriction endonuclease indicated. The digested DNA was electrophoresed on a 0.7% agarose gel, the gel blotted by a bidirectional modification of the Southern procedure<sup>24</sup> and the blot probed with nick-translated pDH3 DNA. The autoradiogram is shown. *b*, DNA from *T. ni* larvae, L, or cultured cells, C, was digested as indicated, electrophoresed, blotted and probed with nick-translated pDH7 DNA. In all cases, 10 µg of DNA were loaded per gel lane. Each hybridization was in 25 ml of 6×SSC (1×SSC = 0.15 M sodium chloride, 0.015 M sodium citrate), 0.1% SDS, 25 mM sodium phosphate, pH 7.0, 1×Denhardt's solution, 100 µg ml<sup>-1</sup> denatured calf thymus DNA for 36 h at 65 °C. The probe (specific activity 2–5 × 10<sup>7</sup> d.p.m. µg<sup>-1</sup>) was at 20 ng ml<sup>-1</sup>. The filters were washed in 2×SSC, 0.1% SDS at 60 °C. Size markers (in kb) were provided by a *Hind*III digest of λ DNA.



**Fig. 3** Direct terminal repetitions in TED. pDH7 DNA was double-digested with *Pst*I and *Hind*III and the material electrophoretically separated on low-melting temperature agarose. The DNAs at 1.425 and 1.025 kb (see Fig. 1b) were cut from the gel, extracted and nick-translated. pDH6, pDH3 and pDH7 DNA were double-digested with *Pst*I and *Hind*III and this material was electrophoretically separated on a 0.9% agarose gel which was bidirectionally blotted to nitrocellulose. The replicas were hybridized to the probes, as indicated. The relevant portion of the ethidium bromide (EtBr)-stained gel as well as that of each autoradiogram is shown. 6, pDH6; 3, pDH3; 7, pDH7. Hybridization conditions were as described in Fig. 2 legend.

either sequence polymorphisms<sup>8,9</sup> or partial copies<sup>10</sup> of this middle repetitive sequence. (In another comparison using a *Drosophila* histone gene clone as probe, the larval and cellular DNAs were identical in both copy number and physical map; data not shown.)

The terminal *Pst*I sites of the FP-DL insert (Fig. 1b) are located at exactly the same distance from the left *Xho*I site and right *Hind*III site of the *Hind*III-K fragment as the *Pst*I site of FP-DS. This is indicative of direct terminal repeats in FP-DL and a relationship between FP-DS and FP-DL. Sequence homology within and between FP-DS and FP-DL was observed directly by hybridization. pDH7 was digested with *Pst*I and *Hind*III to give two DNAs of 1.425 and 1.025 kb, covering the region of interest. These were used in turn as probes of blots of *Pst*I/*Hind*III-double-digested pDH7, pDH3 and pDH6. The 1.425 kb fragment is homologous to the 2.2 kb fragment (left-end) of pDH3 and pDH6, demonstrating both the presence of direct terminal repeats in FP-DL and that FP-DS contains a sequence homologous to the terminal repeat of FP-DL (Fig. 3). The 1.025 kb fragment is homologous to itself and to the portion of pDH6 containing *Hind*III-K sequences (right-end). The 1.025 kb fragment does not hybridize to the 0.95 kb *Pst*I fragment at the left end of the FP-DL insert (present in pDH3) because the *Pst*I site is at the far right of the terminal repeat and there is not a sufficiently long region for hybridization.

The insertion present in FP-DL is thus a 7.3 kb sequence with direct terminal repeats of 0.27 kb; FP-DS contains one of these terminal repeats. FP-DL apparently arose by a transposition of the 7.3 kb sequence from the host genome to a viral genome during serial passage. Furthermore, the endogenous host sequence has moved and amplified with only slight alteration in the cell line we use. These data indicate that it is a transposable element, probably of the *copia*-like class. *Copia* is a middle repetitive sequence of 5 kb found in *Drosophila melanogaster*, containing direct terminal repeats of 0.276 kb (ref. 11). Evidence for the mobility of this class of sequences (as well as that of other mobile sequences; see, for example, ref. 12) includes the demonstration of substantial variation in its genome location, depending on the strain examined, and an amplification of the sequence in cell lines as compared with embryos<sup>10,11,13,14</sup>. We have designated the FD-DL insertion as

transposable element D (TED). The direct terminal repeats at its ends, since one exists alone in FP-DS, have been designated ID sequences. Together FP-DL and FP-DS constitute the FP-D mutant virus.

The small amount of FP-DS in FP-D remains to be explained. We favour a model in which recombination between the direct terminal repeats of replicating FP-DL continually generates FP-DS, making it impossible to plaque-purify FP-DL free of FP-DS, and yielding the FP-D genotype. Evidence for this will be presented elsewhere. We have not established whether the insertions present in FP-DL and FP-DS play a part in the FP phenotype of FP-D, although transcription experiments underway suggest that they do (unpublished observations). The phenomenon reported here is probably one of many ways FP mutants of NPVs can be generated since other FP mutants contain no discernible insertion<sup>6</sup> and, conversely, insertions are found in phenotypically wild-type variants<sup>15</sup>.

We believe this to be the first report of a *copia*-like transposable element in a metazoan besides *Drosophila*. It may be

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significant that both are in insects. It is also the first example, other than the Ty1 element of yeast<sup>10</sup>, in which a terminal repeat can be found free of the main body of the transposable element. Finally, it is the first report of a transposable element integrated into a eukaryotic viral genome. The parallel has been drawn between proviruses and transposable elements<sup>16–18</sup>, and the origin of proviruses from such elements has been logically proposed<sup>16,17</sup>. If this is the case, it seems particularly noteworthy that such an element should be found integrated into a viral genome. This 'stowaway' manoeuvre might be expected of selfish<sup>19,20</sup> (or parasitic<sup>21</sup>) DNA and may have a role in the inter-organismal movement of these sequences as well as their evolution and that of NPVs.

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## Crystal structure of a microbial ribonuclease, RNase St

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The existence of a group of microbial RNases that hydrolyse 3', 5'-phosphodiester linkages of RNA with a mechanism similar to that of pancreatic RNase A has long been known<sup>1</sup>. There is a rather poor homology between the six known primary sequences of these enzymes (Table 1), but the alignment around the possible functional residues may be systematic. There seems to be no homology at all between the primary sequences of these RNases and those<sup>2</sup> of pancreatic RNases. The three-dimensional structure of RNase has been determined only for one member of the latter group, bovine pancreatic RNase A<sup>3,4</sup> and its derivative, RNase S<sup>5,6</sup>. Here we report the three-dimensional structure of a microbial ribonuclease, RNase St, determined by the isomorphous replacement method. Both the polypeptide chain folding (reported earlier)<sup>7</sup> and the active site environment are completely different from those of RNase A (or S), while they bear partial resemblance to those of barnase<sup>8</sup> (a ribonuclease from *Bacillus amyloliquefaciens*).

RNase St is an extracellular ribonuclease guaninenucleotido-2'-transferase (cyclizing, EC 2.7.7.26) from *Streptomyces erythreus* isolated by Tanaka<sup>9</sup>, characterized<sup>10–12</sup> and sequenced<sup>13</sup> by Yoshida *et al.* It has a single polypeptide chain of 101 amino acid residues with one disulphide bond (4–54). Since the original study<sup>13</sup>, the N-terminal residue has been found to be Gln instead of Glu and Gly 26 has been shown to be non-existent<sup>14</sup> (K. Miyamoto, H. Matsuo, K. Narita & N. Y., unpublished chemical observation; the latter fact was also confirmed by the present X-ray study) making the present numbering after Tyr 26 smaller

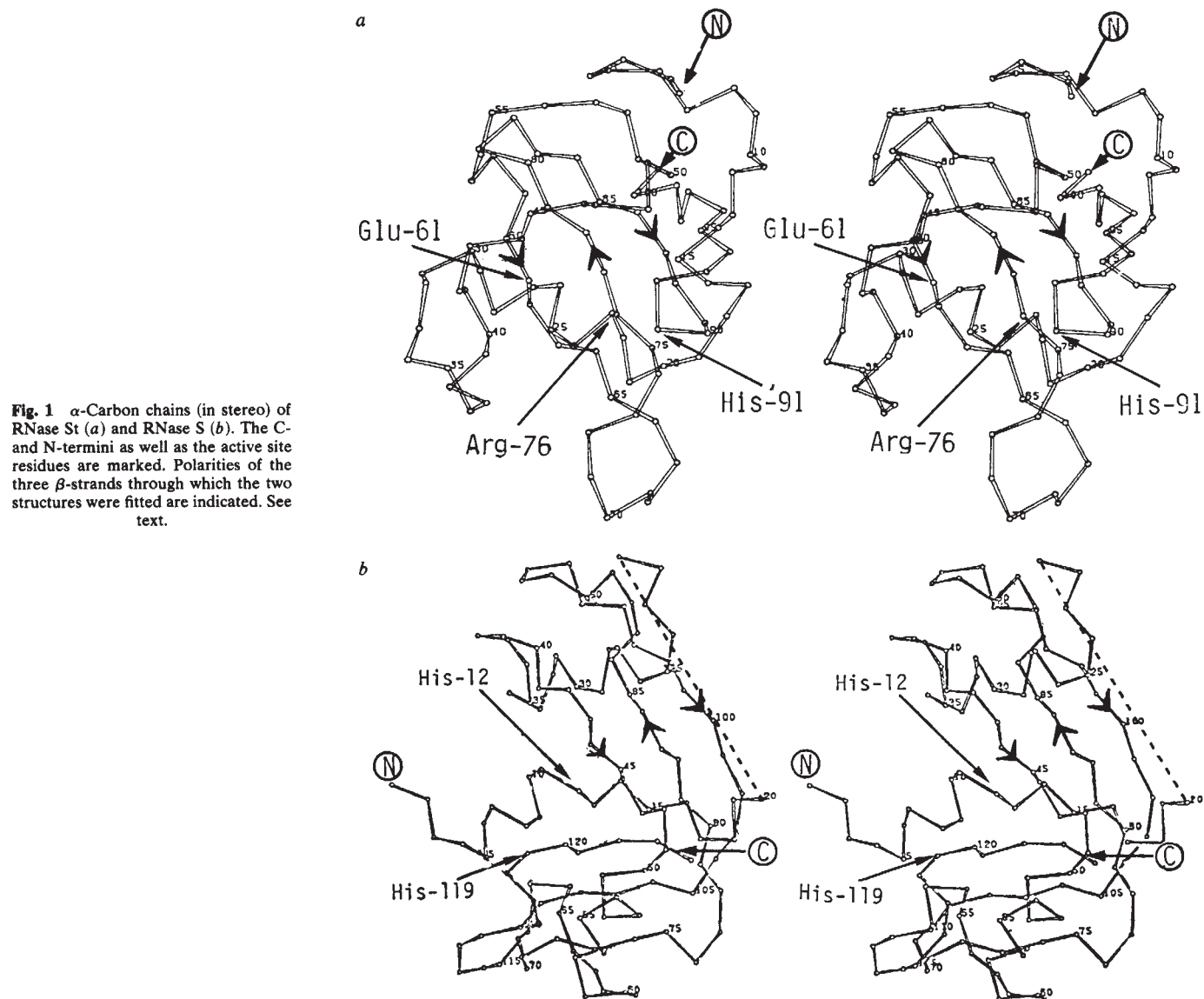
by one. Crystallization and crystallographic characterization have been reported elsewhere<sup>15</sup>. The crystal structure was solved at 2.5 Å resolution by the isomorphous replacement method using KAu(CN)<sub>4</sub>, C<sub>6</sub>H<sub>5</sub>Hg(OCOCH<sub>3</sub>), UO<sub>2</sub>(AcO)<sub>2</sub>, K<sub>3</sub>IrCl<sub>6</sub> and (NH<sub>4</sub>)<sub>2</sub>HgCl<sub>4</sub> derivatives for which the ratios (r.m.s.-heavy atom structure factor/r.m.s. lack-of-closure error) were (31.6/17.1), (23.2/11.9), (17.6/12.2), (51.9/34.6) and (24.0/18.9), respectively. The quality of the resultant electron density map was such that, for some tyrosines, bumps corresponding to each non-hydrogen atom of the phenol ring could be seen. Details of the structure analysis will be published elsewhere (K. Iwahashi *et al.*, manuscript in preparation).

The structure consists of a three-and-half turn  $\alpha$ -helix (residues 19–32) and an antiparallel  $\beta$ -sheet consisting of  $\beta_1$ (58–63),  $\beta_2$ (74–81) and  $\beta_3$ (83–91) strands (Fig. 1a). There are two large twisted loops, 46–57 and 65–73, the latter being very flexible. In addition, there are two interstrand interactions, one between the  $\beta_1$ -strand and the 39–45 chain segment and the other between the 96–100 and the 10–15 chain segments. The simplified topology/packing diagram indicates that RNase St belongs to class III or the  $\alpha + \beta$  type of Levitt and Chothia<sup>16</sup>. Figure 1b is the  $\alpha$ -carbon chain of RNase S<sup>5,6</sup> oriented to optimize its fit of the antiparallel  $\beta$ -sheet to the corresponding sheet of RNase St by a least-squares procedure<sup>17</sup> (see Fig. 1 legend). 'Homology' between the two RNases seems merely topological with respect to only the  $\beta$ -sheet region and thus cannot be regarded as indicating an evolutionary relationship.

The structures of RNase St and barnase<sup>8</sup>, on the other hand, are highly correlated, the  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -strands described above corresponding to the  $\beta_2$ -,  $\beta_3$ - and  $\beta_4$ -strands of barnase, respectively. The spatial arrangements of the most probably functional residues (Glu 61, Arg 76 and His 91 in RNase St; Glu 73, Arg 87 and His 102 in barnase) relative to the three-stranded  $\beta$ -sheets are similar to each other. However, the two structures are apparently unrelated to each other at the N-terminal 35 residues, the region where the alignment of the primary sequences is also ambiguous. It seems that the spatial arrangement of the first (rather than the second)  $\alpha$ -helix (residues 6–18) of barnase corresponds roughly to that of the only  $\alpha$ -helix of RNase St. However, the unambiguous alignment of the corresponding primary sequences with each other seems

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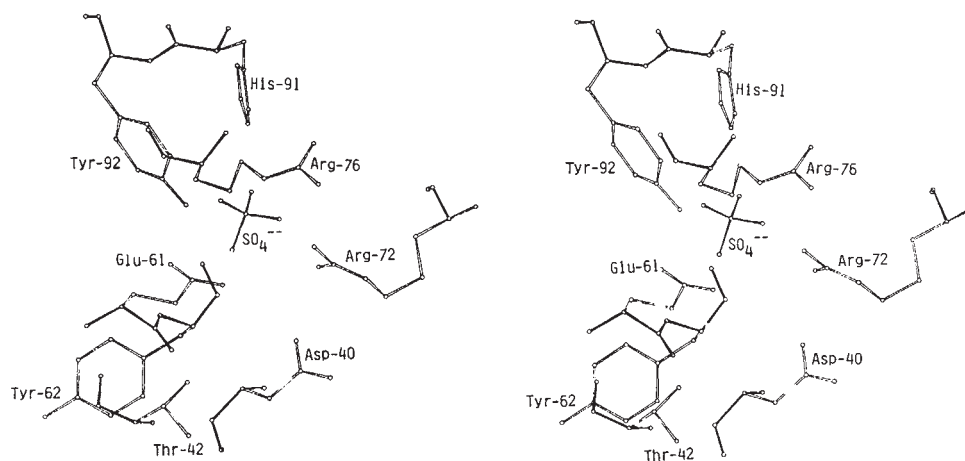


impossible. We predict that the N-terminal unique sequence is due to a distinct exon.

Around the putative active site, we found a large isolated peak too large to be a water molecule. By analogy with RNase S<sup>5,6</sup>, this was assigned to a sulphate anion contained in the crystallization medium occupying the binding site (the  $p_1$  site in the notation for RNase A (or S)<sup>6</sup>) for the phosphate group of the substrate. As shown below, the assumption seems sufficiently reasonable, although we could not prove this directly by obtaining difference Fourier maps for substrate analogues.

Attempts to introduce 3'-GMP or 3'-IMP in the active site failed because, in the present crystal, it is blocked by a neighbouring molecule.

The sulphate anion sits in a strongly polar pocket (Fig. 2). The following atoms can form hydrogen bonds with one of the oxygens of the sulphate: N<sup>n1</sup> of Arg 72, N<sup>e1</sup>, N<sup>n2</sup> of Arg 76, N<sup>e2</sup> of His 91 and O<sup>n</sup> of Tyr 92. Moreover, O<sup>e1</sup> and O<sup>e2</sup> of Glu 61 are located very close to an oxygen of the sulphate anion. This geometry and assumption that the sulphate is bound to the  $p_1$  site for the substrate are completely consistent with



**Fig. 2** The active site residues and a bound sulphate anion in RNase St shown in stereo. See text.



Table 1 Primary sequences of microbial RNases

|                |                                                                   |
|----------------|-------------------------------------------------------------------|
| U <sub>2</sub> | C N I P E S T N C G N V Y S N - D D I N T -                       |
| Ms             | E S C E Y T C G S T C Y W S - S D V S A -                         |
| T <sub>1</sub> | A C D Y T C G S N C Y S S - S D V S T -                           |
| St             | Q A P C G D T S G F E E V R L A D L P                             |
| Bi             | A V I N T F D G V A D Y L I R Y K R L P N D Y I T K S Q A S A L   |
| Ba             | A Q V I N T F D G V A D Y L Q T Y H K L P N D Y I T K S E A Q A L |
| U <sub>2</sub> | - - - A I Q G A L D D V A R P D - G D N Y P H Q Y Y D - E A S D Q |
| Ms             | - A K A K G Y S L Y E S G E T I - - D D Y P H E Y H D Y E G F D F |
| T <sub>1</sub> | - A Q A A G Y Q L H E D G E T V G S N S Y P H K Y N N Y E G F D F |
| St             | P E A T D T Y E L I E K G G P Y P Y P E D G T V F E N R E G L P   |
| Bi             | G W V A S K G D L A E V A - P G - K S I G C D V F S N R E G R L P |
| Ba             | G W V A S K G N L A D V A - P G - L S I G C D I F S N R E G K L P |
| U <sub>2</sub> | I T L C C G P G S W S E F P L V Y N G P Y Y S R R N Y V S G P     |
| Ms             | P V G T - - - S Y Y - E Y P I M S D Y D V Y T - - - G S P G A     |
| T <sub>1</sub> | S V S S - - - P Y Y - E W P I L S S G D V Y S G - - - P G S G A   |
| St             | D C A E - - - G Y Y H E Y T V - - - K T P S G - - - D D R G A     |
| Bi             | S A G S - - - R T W R E A D I - - - N Y V S G - - - F - - - R N A |
| Ba             | G K S G - - - R T W R E A D I - - - N Y T S G - - - F - - - R N S |
| U <sub>2</sub> | D R V I Y Q T N T G E F C A T V T H T G A A S Y D G F T Q C S     |
| Ms             | D R V I F D G D D - E L A G V I T H T G A A G G D D V A C S S S   |
| T <sub>1</sub> | D R V V F N E N N - Q L A G V I T H T G A - S G N N F V E C T     |
| St             | R R F V V G D G G - E Y F Y T E D H Y E - - - S F R L T I V N     |
| Bi             | D R L V Y S S D W - L I Y K T T D H Y A - - - T F T R I R         |
| Ba             | D R I L Y S S D W - L I Y K T T D H Y Q - - - T F T K I R         |

Residues that are homologous in three or more sequences are boxed. Somewhat arbitrary homology relationships, T=S; Y=F; V=L; I; D=N=E=Q, were assumed. Residues for which catalytic function has been implied are indicated by arrows. Most of these have been originally deduced for RNase T<sub>1</sub> (refs 20, 21, 31). Sources and base specificities are as follows: U<sub>2</sub>, *Ustilago spheerogena*, purine-specific<sup>32,33</sup>; Ms, *Aspergillus saitoi*, nonspecific (G>A>C>U)<sup>34</sup>; T<sub>1</sub>, *Aspergillus oryzae*, guanine-specific<sup>35</sup>; St, *Streptomyces erythreus*, guanine-specific<sup>33</sup>; Bi, *Bacillus intermedius* 7P, mainly purine-specific<sup>36</sup>; Ba, barnase (*Bacillus amyloquelificans*)<sup>37</sup>. The alignments of Ms with T<sub>1</sub> and Bi with Ba can be done unambiguously forming the T<sub>1</sub> group and the barnase group respectively. U<sub>2</sub> and St are closer to the T<sub>1</sub> group and the barnase group, respectively, but the alignment for the N-terminal 30 residues is not clear in either case. The alignment of the T<sub>1</sub> group with the barnase group cannot be done unambiguously for the first 40 and the last 6 residues (in barnase numbering).

other observations<sup>18,19</sup>. According to hydrogen-tritium exchange and <sup>1</sup>H NMR studies<sup>18</sup>, on addition of 3'-GMP, the pK<sub>a</sub> of His 91 increases by about 1.2 units while that of His 60 is virtually unaffected. In the present model His 60 is located opposite to the active site across the β-sheet, excluding any possibility of its functional role. Unusual reactivity of a glutamate residue to iodoacetate generally found among microbial RNases has been confirmed<sup>19</sup> and identified<sup>19</sup> as Glu 61 in RNase St. Coexistence of 2' (3')-GMP was found most effective in inhibiting the carboxymethylation<sup>19</sup> which completely inactivates the enzyme. Glu 61, Arg 76 and His 91 are invariant among the six microbial RNases and evidence for the functional significance of the corresponding residues in RNase T<sub>1</sub> (Glu

58, Arg 77 and His 92) has accumulated<sup>20-23</sup>. The corresponding residues in barnase (Glu 73, Arg 87 and His 102) were found to cluster in one region of the molecule<sup>8</sup>. It seems most likely that the carboxylate of Glu 61 and the imidazole of His 91 in RNase St, as well as the corresponding groups in other microbial ribonucleases, play the parts of His 12 and His 119 of RNase A (or S). This scheme is similar to that proposed earlier for Glu 58 and a histidine residue of RNase T<sub>1</sub> (Takahashi<sup>24</sup>) except that the latter residue is specified. Arg 76 and Arg 72 of RNase St may be substitutes for Lys 7 and Lys 41 of RNase A (or S).

In RNase A, the presence of an aspartyl residue (Asp 121) apparently enhancing the functional role of His 119 has been noted from a theoretical point of view<sup>25</sup> and apparently confirmed in the refined structure of RNase A<sup>26</sup>. However, in RNase St, we see no hydrogen-bonded interactions involving the imidazole ring of His 91 and an acidic side chain. In fact, the imidazole ring is very close to the guanidine group of Arg 76. This fact rather favours another theoretical study<sup>27</sup> made for RNase A in which a mere steric role for positioning the substrate was assigned to Asp 121, although the mechanisms to activate histidines might be different for RNases A and St. In this connection, we should emphasize a possible role played by Tyr 92 of RNase St which has never been described. The phenolic oxygen O<sup>n</sup> clearly interacts with O<sup>r1</sup> of Glu 61, the distance being 2.6 Å, although the angle C<sup>δ</sup>-O<sup>r1</sup>-O<sup>n</sup> (~100°) is not ideal for a hydrogen bond. This residue is preserved in RNase Bi and barnase while it is threonine in RNase T<sub>1</sub>, U<sub>2</sub> and Ms. Any further argument must await the elucidation of the three-dimensional structure of RNase St complexed with a substrate analogue and that of RNase T<sub>1</sub> which was first crystallized by the present group<sup>28</sup> and is reported to be under structure analyses<sup>29,30</sup>.

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**Note added in proof:** Since submission of this paper, we have learnt of the structure determination at 2.5 Å resolution of ribonuclease T<sub>1</sub> (ref. 38) in which the three functional residues mentioned above seem to be arranged similarly.

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